



1976

# An Ultrastructure Study on the Effect of Adrenalectomy and Cortisone on the Preameloblasts of the Rat Maxillary Incisor

Maynard James Marshall  
*Loyola University Chicago*

## Recommended Citation

Marshall, Maynard James, "An Ultrastructure Study on the Effect of Adrenalectomy and Cortisone on the Preameloblasts of the Rat Maxillary Incisor" (1976). *Master's Theses*. Paper 2804.  
[http://ecommons.luc.edu/luc\\_theses/2804](http://ecommons.luc.edu/luc_theses/2804)

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact [ecommons@luc.edu](mailto:ecommons@luc.edu).



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License](https://creativecommons.org/licenses/by-nc-nd/3.0/).  
Copyright © 1976 Maynard James Marshall

AN ULTRASTRUCTURE STUDY ON THE EFFECT OF ADRENALECTOMY AND  
CORTISONE ON THE PREAMELOBLASTS OF THE RAT MAXILLARY INCISOR

by

Maynard James Marshall, B.S., D.D.S.

A Thesis Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Science

June

1976

## LIFE

Maynard James Marshall was born April 19, 1935 in Gillingham, Wisconsin.

He was graduated from Richland Center High School, Richland Center, Wisconsin in June, 1953. He then attended the University of Wisconsin, Madison, from 1953 to 1957 and received the Degree of Bachelor of Science in Microbiology. He then proudly served his country in military service and upon receiving his honorable discharge entered Marquette University Dental School. He was graduated with the degree of Doctor of Dental Surgery in June, 1964.

He was married to the former Linda R. Newman in Dugway, Utah, June 17, 1961. He has two sons, James Edward and John Newman.

He practiced general dentistry in Madison, Wisconsin from July, 1964 to June, 1972, after which time he began his graduate studies in the Department of Oral Biology and postgraduate studies in the Department of Orthodontics, Loyola University School of Dentistry, Maywood, Illinois.

## ACKNOWLEDGEMENTS

The author wishes to thank and express his sincerest appreciation and gratitude to the following:

Michael L. Kiely, Ph.D. my advisor, for his ever present help, direction, guidance and understanding throughout the period of my graduate training.

Also my sincere thanks to Dr. Hal McReynolds for his helpfulness, suggestions and advice.

I am also grateful to other members of the Anatomy Department for their suggestions, help and encouragement. I am especially grateful to Mrs. Letta Prevas, for her cooperation and perserverance in the typing of this manuscript.

John V. Madonia, D.D.S., Ph.D., Donald C. Hilgers, D.D.S., Ph.D., and James Jensen, D.D.S., M.S.D. for the opportunity to pursue graduate studies.

A very special appreciation to the technicians of the Pathology Department, especially Mrs. Birute Prapuolenis, for without her help and contributions this study would have been impossible.

And finally, to my wife Linda, my sons James and John for their love, devotion and understanding during the past two years.



# TABLE OF CONTENTS

	<u>Page</u>
I. INTRODUCTION . . . . .	1
II. REVIEW OF LITERATURE . . . . .	3
A. Mechanisms of Tooth Eruption . . . . .	3
B. Effect of Cortisone on Tooth Eruption. . . . .	7
C. Histological Changes due to Adrenalectomy and/or Cortisone Administration . . . . .	9
D. Electron Microscopy. . . . .	12
1. Developing Cells of Incisor. . . . .	12
2. Differentiating Cells of Incisor . . . . .	15
3. Hormonal Effects on Tissue . . . . .	18
III. MATERIALS AND METHODS. . . . .	20
IV. EXPERIMENTAL RESULTS . . . . .	23
A. Observations on Control Animals. . . . .	23
B. Observations on Saline Treated Adrenalectomized Animals. . . . .	25
C. Observations on Cortisone Treated Adrenalectomized Animals . . . . .	26
V. DISCUSSION . . . . .	27
VI. SUMMARY AND CONCLUSIONS. . . . .	32
VII. LITERATURE CITED . . . . .	35
VIII. LEGEND, FIGURES AND PLATES . . . . .	42

## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1	A low power photomicrograph of a sagittal section of a maxillary incisor showing the cervical labial loop in a young normal rat . 43
2	The cervical loop of a maxillary incisor at higher magnification 43
3	A high power photomicrograph showing the major cell layers of the cervical loop of the rat incisor . . . . . 44
4	An oil immersion light photomicrograph showing the magnification of the cell layers shown in Figure 3 . . . . . 44
5	Low power electron micrograph showing the preameloblasts of a control rat. . . . . 45
6	Electron micrograph from a control animal showing the basal lamina separating the preameloblasts and preodontoblasts . . . . 46
7	Electron micrograph from a control rat showing the width and density of the basal lamina. . . . . 47
8	Electron micrograph of preameloblasts in a control rat showing the presence of mitotic figures. . . . . 48
9	Electron micrograph of cytoplasm of normal preameloblasts with well developed Golgi complex . . . . . 49
10	Electron micrograph of a control rat showing large oval nuclei and a mitotic figure . . . . . 50
11	Electron micrograph of tight junction and of normal preameloblasts . . . . . 51
12	Electron micrograph of several cell organelles in control animals. . . . . 52
13	Electron micrograph of differentiated, secreting ameloblasts and odontoblasts . . . . . 53
14	Low power electron micrograph of an untreated adrenalectomized rat showing many intercellular spaces as compared to Figure 5. . 54
15	Electron micrograph of a preameloblast nucleus from an adrenalectomized rat . . . . . 55

# LIST OF FIGURES (continued)

<u>Figure</u>		<u>Page</u>
16	Electron micrograph of nuclear inclusions from an adrenalectomized animal . . . . .	56
17	Electron micrographs of three ameloblast nuclei showing nuclear inclusions . . . . .	57
18	Electron micrograph of nuclear inclusions and bizarre mitochondria from an adrenalectomized rat. . . . .	58
19	Electron micrograph of lysosomes and vacuoles from an adrenalectomized rat . . . . .	59
20	Electron micrograph showing some slightly swollen mitochondria cristae from an adrenalectomized rat . . . . .	60
21	Electron micrograph of an elongated mitochondrion from an adrenalectomized rat . . . . .	61
22	Electron micrograph of the basal lamina from an adrenalectomized rat. . . . .	62
23	Electron micrograph of preameloblasts and preodontoblasts of a cortisone treated animal . . . . .	63
24	Electron micrograph of preameloblasts of a cortisone treated animal . . . . .	64
25	Electron micrograph of bizarre shaped mitochondria from cortisone treated animals. . . . .	65

## INTRODUCTION

It is well known that the incisor teeth of the albino rat continue to erupt throughout life and that this eruption is under hormonal control. The adrenal cortical steroid, cortisone has been shown by numerous experiments to increase the eruption rate of the incisor. It has also been shown that the eruption rate of an adrenalectomized rat is severely inhibited. Thyroidectomy and hypophysectomy show similar results and further enhance the theory of hormonal influence on rat incisor eruption.

Studies have shown that cortisone causes an increase in the number of mitosis in the cells of the cervical loop of the rat maxillary incisor during certain periods of the 24 hour cycle. Further, an adrenalectomized rat will show a marked reduction in this proliferative activity, as evidenced by less <sup>3</sup>H-thymidine labelled cells, but upon injection of cortisone a significant increase in the number of these cells is noted. It is postulated that the primary action of cortisone may be a stimulation of DNA replication in preparation of cell division. However, the actual manner in which cortisone promotes growth of the incisor is still to be determined. However, it can be concluded that cortisone does promote the rate of incisor eruption by stimulating proliferation of formative tissues at the apex of this organ.

In recent years, the electron microscope has been used somewhat extensively to study the proliferating tissues of the incisor. This is especially true of the odontogenic cells of the enamel organ proper. The normal cells have been quite well observed with the electron microscope.

Although studies have been made concerning adrenalectomy and cortisone effects on the tissues of the cervical loop, none have been performed at the electron microscopic level. With the use of the electron microscope we propose to study the effect of adrenalectomy and subsequent replacement with cortisone on the ultrastructure of the proliferating loop tissues of the rat incisor. With this investigation we hope to gather specific information with respect to the influence of cortisone on some of the intracellular events taking place and any observable changes or alterations occurring in these proliferating tissues.

## REVIEW OF LITERATURE

### A. Mechanism of Tooth Eruption

Many investigators have presented data supporting the concept that cell division is the basic cause of tooth eruption. However, there has been considerable disagreement over the years as to the factors actually responsible for this process. As early as 1835 Thomas Bell suggested that eruption of teeth was due to the elongation of the roots. Constant (1900) felt Bell's theory was not feasible and proposed on the basis of anatomical studies that the eruptive forces were due to blood pressure on periapical vascularity. Among the first to propose cell division as the cause of tooth eruption was Addison and Appleton (1915). Therefore, cellular proliferation at the basal end of the tooth and the tissue vascularity - blood pressure theories have been the two major areas of study in regard to tooth eruption. Also, recently, growth of the periodontal ligament has been suggested as a force in incisor eruption.

Massler and Schour (1941) reviewed many theories proposing causes of eruption. These theories included: (1) growth of the root, (2) growth of dentin and pulpal constriction, (3) growth of the periodontal membrane and alveolar process, (4) pressure from muscular action on the alveolar process, (5) resorption of alveolar bone, (6) pressure from cellular proliferation, and (7) pressure from the vascular bed of the pulp and periapical tissues. It was their conclusion that the eruptive force is due to the vascularity of the periapical tissue but not of the pulp. However, they were careful to state that

many factors could be involved in the eruptive force. Thus they concluded that cellular proliferation could certainly be one of the factors involved in tooth eruption.

Sicher (1942) felt that the proliferating pulp cells are the cause of eruption. His studies were based on morphological studies on human teeth and the rat incisor. He suggests that this pulpal growth occurs in a restricted zone at the basal end of the tooth just above the epithelial diaphragm. He described a "hammock" ligament upon which the tooth rests. It is anchored in the periodontal ligament and bone and separates the periapical tissues and the pulpal tissues. This ligament supposedly acts as a cushion protecting the basal bone, keeping it from being resorbed and also at the same time transmitting the pressure from pulpal proliferation in a coronal direction causing eruption.

Experiments by Scott (1953) Sturman (1957) and Eccles (1965) all support this hypothesis. However Ness and Smale (1959) and Main (1965) were both unable to find any evidence of a hammock ligament in their studies utilizing dogs, cats, sheep, rabbits, mice, and rats as described by Scott and Sicher.

In 1941 Herzberg and Schour removed the pulps from the lower incisors of albino rats. They found no change in the eruption rate of the teeth. They also excised Hertwigs sheath surgically and again found no change in the eruption rate. Thus it was their conclusion that the growth of dentin, Hertwig's sheath and the pulp was not necessary for tooth eruption. In (1957) Bryer repeated the experiments of Herzberg and Schour and obtained different results. He noted that there was initially a decrease in the eruption rates but a slow gradual recovery did occur.

Ulmansky and Shapiro (1964) found that the greater the damage done to pulp the greater the inhibition of eruption. Thirty nine rats were divided into 3 groups and had their lower right incisor amputated at the gingival level with the lower left tooth serving as the control. The first group had no further trauma, but pulpal damage was inflicted on the second group by placing a dental reamer in the pulp cavity; and group 3 received permanent irritation by the insertion of a silver point in the canal. A complete inhibition of eruption was often noted in this last group.

Taylor and Butcher (1951) performed vascular ligations on major arteries and interrupted sympathetic innervation in the rat and found the rate of eruption is not sensitive to considerable changes in blood flow. Sympathectomy seemed to have no effect on eruption.

In 1957 Bryer repeated the experiment conducted by Taylor and Butcher in regard to sympathectomy. He found that this procedure increased blood flow and eruption rate as a result of the removal of vasoconstrictor tone. He stressed the importance of pulpal vascularity and periodontal tissues as factors of eruption.

Ness (1964) reported that blood pressure is the primary force responsible for tooth eruption. He later changed his ideas and reported that he supported the hypothesis that fibroblasts located in the periodontal tissue exert tension along their length which provide the forces necessary for the eruption of the rat incisor.

Main and Adams (1966) conducted experiments using hypotensive (guanethidine and hydralazine) and antimitotic (demecolone and triethylene) drugs on the eruption rate of the rat incisor. It was their conclusion that there was no



direct relationship between the rate of eruption and blood pressure or between this rate and the cellular activity of the pulp. They suggested the periodontal ligament as the most likely tissue involved in the mechanism of tooth eruption.

A number of investigations have reported that the dental follicle and/or periodontal ligament may play a role in the eruptive process. Hoffman and Gillette (1964) in their study on the developing hamster molar; concluded the pulpal cells and the periodontal tissues were responsible for eruption. Also in 1967, Gowgiel felt it was the growth of the follicular sac which caused eruption. He reported that irradiation of the teeth allowed crowns to erupt even though root formation was destroyed. When the root did not form, the follicular sac failed to differentiate into the periodontal membrane but remained attached to the cementum of the crown. Using 3-H thymidine he demonstrated by the increased tagging of follicular cells that the follicular sac continued to grow. Thus it was concluded that tooth eruption was due to the growth of the tooth follicle. His study also suggests that because there was no root formation, dentinal and pulpal growth could be eliminated as eruptive forces. Also because of the sclerosis of the blood vessels due to irradiation the vascular theory of tooth eruption could be seriously questioned.

Thomas (1964 and 1967) reports that collagen maturation is an important factor in the relationship of eruption with bone growth and tooth development. During tooth development, the fibers of the follicle are reorganized and rearranged causing intermolecular and intramolecular cross-linkages within the periodontal ligament which produced tension in its oblique fibers causing a pulling effect of the tooth in a coronal direction.

Berkowitz (1972) used normal and root-resected lower rat incisors to study

the effects of anti-mitotic drugs upon eruption. The drugs caused a decrease in the eruption rate of both the normal and root-resected incisors. From his studies he concluded that the periodontal ligament is probably the most important factor in the eruptive process.

Not only is there much evidence to conclude that mitotic activity is responsible for tooth eruption, but the administration of cortisone has been shown to bring about a stimulation of DNA synthesis and mitosis in the enamel organ and adjacent pulpal tissue in the incisor of adrenalectomized and normal rats (Domm and Kiely, 1968 and Kiely and Domm, 1973). They have concluded that cell proliferation must be taken into consideration when discussing the factors which are involved in the control and mechanism of tooth eruption.

#### 8. Effect of Cortisone on Tooth Eruption

Numerous previous investigations have quite conclusively shown that cortisone plays an active role in the eruptive process of the rat incisor. Administration of this steroid results in an increase in the normal eruption rate. Parmer, Katonah and Angrist (1951) noted that after cortisone injection to newborn rats, the incisors erupted 2.5 days sooner than did the controls. Domm and Marzano (1954) found that cortisone increased the eruption rate of the incisor in both young and adult rats. Also a constant ratio was maintained for upper to lower incisor rates in both males and females. In the newborn rat, the incisor erupted earlier than normal and in both sexes the rates were greatly increased with the cortisone administration, while upper to lower incisor ratios remained constant. In this same investigation hypophysectomized rats showed a decrease in the eruption rate of the incisors in both sexes. However, when these animals were given cortisone the rate of eruption increased,

but with a disturbance of the upper to lower incisor ratio. In an earlier work, Schour and Rogoff (1936) have shown that adrenalectomy causes a retardation in incisor eruption as well as an over calcification of the dentin. Domm and Leroy (1955) noted that the incisors of newborn rats erupted at an increased rate when cortisone was given to the pregnant mother, to the newborn rats or to the fetus in utero.

Garren and Greep (1967) and Domm and Wellband (1960) have shown that cortisone also accelerates the inhibited rate of incisor eruption in adrenalectomized rats. They also reported reduction in the eruption rate following thyroidectomy and thyroadrenalectomy. Again cortisone therapy increased the eruption rate of these animals.

Wellband and Domm (1964) observed a further increase in the rate of eruption of rat maxillary incisors that were periodically amputated, following the administration of cortisone.

A study of Goldsmith and Ross (1956) showed an increased alkaline phosphatase and RNA content of the ameloblasts and odontoblasts in the incisor of fetal rats treated with cortisone. Cortisone also increased the glycogen content of the stratum intermedium. The incisors of postnatal rats revealed an early tissue differentiation with cortisone treatment. Later in development, an overgrowth of alveolar bone, degeneration and ameloblasts and disorganization of the periodontal connective tissue was noted under the influence of cortisone.

In the young rat incisor, Domm and Kiely (1968) observed that an increase in the number of mitotic figures of the cervical loop cells resulted due to cortisone administration. Kiely (1967) observed that important factors in the response of tissues to cortisone are dosage, frequency, and duration of treat-

ment. Kiely and Domm (1973) also reported that with the administration of cortisone there is an increase in the number of 3H-thymidine labelled cells in the cervical loop tissues of adrenalectomized rats.

Corticosteroids have been shown to have a wide pharmacological action and are involved in many physiological functions. As presented by Goodman and Gilman (1970) it is shown they are involved in the metabolism of carbohydrates, proteins, fats and purines. They are also involved in water and electrolyte balance and the functional capacities of the skeletal muscle, cardiovascular system, kidneys and the nervous system.

Although cortisone seems to stimulate mitotic activity causing an increase in eruption rates in the incisor, it is interesting to note that in a study by Dustin (1963) it was noted cortisone has been found to completely inhibit cell division in some tissues. Also in some tissues there was no effect.

It is apparent from the literature that there is a general agreement regarding the observation that cortisone does enhance the eruption of the rat incisor. However, the reasons for this effect must be further investigated as they are not completely understood at the present time.

#### C. Histological Changes due to Adrenalectomy and/or Cortisone Administration.

The literature contains a number of reports in which the effects of cortisone and adrenalectomy on oral tissues have been studied and described histologically. After the treatment of mice with cortisone, Glickman, Stone and Chawla (1953) have found alveolar bone to be reduced in height and osteoporotic with decreased numbers of osteoblasts. They also have found a reduction in amount of osteoid matrix and fibrous transformation of the periodontal space with diminished numbers of fibroblasts and collagen fibrils and degenerative changes.

Applebaum and Seelig (1955) found loss of supporting bone tissue around rat molars after treatment with cortisone. They also reported vascular changes in the pulp of these teeth; the major change being severe dilatation of the vessels. In this same study, adrenalectomy was reported to also cause very dilated blood vessels as well as hemorrhage in the pulp.

In the early work of Schour and VanDyke (1932) hypophysectomy not only caused a retardation of tooth eruption, but it also brought about an over calcified state in the dentin. This work also demonstrated that hypophysectomy caused a retardation of eruption and cessation of amelogenesis while growth hormone overcame this dental defect.

In a study by Baume et al. (1953 and 1954) it was reported that following hypophysectomy there were symptoms of disorganization of the enamel epithelium, disturbances of calcification and odontoblastic agenesis. They found that growth hormone therapy restored all histological features but one to normal. The one exception was amelogenesis upon which the growth hormone had no effect.

Goldsmith and Stahl (1953) studied the effects of cortisone treatment on the supporting dental structures in the rat. They found an increase in the amount of alveolar bone and a narrowing of the marrow spaces when cortisone was given at a 3 mg dosage over a period of a year. They also observed a disorganization of the periodontal fibers in these animals.

Goldsmith and Ross (1956) treated post-natal rats with cortisone and found premature histological differentiation and eruption. Later in treatment, they also observed overgrowth of the alveolar bone, degeneration of the ameloblastic layer and disorganization of the periodontal connective tissue.

Shklar (1963) reported osteoporosis in alveolar bone and a decrease in the

density of the periodontal ligament in the teeth of young, adrenalectomized rats. Also, the gingival epithelium and odontoblasts were atrophied with a definite decrease in osteoblastic activity.

Stallard (1962) observed in animals receiving daily injections of cortisone, alveolar bone resorption, disorganization of the periodontal membrane and a breakdown of sub-epithelial connective tissue.

Kiely (1964) reported that injections of cortisone twice daily over a two day period was capable of increasing the mitotic activity of the stratum intermedium, the preameloblasts, preodontoblasts, and adjacent pulp in the upper rat incisor.

Wellband (1961) observed that hypophysectomy resulted in hypoplasia of the enamel organ. There appeared to be a loss in cellularity of the stratum intermedium and stellate reticulum. However, little change was observed in the ameloblasts. There was also a marked decrease in the number and size of pulpal blood vessels which resulted in a more cellular appearance of the pulp. The administration of 1.5 mg of cortisone to hypophysectomized rats produced little or no histological change in the enamel organ when compared with controls.

Anneroth and Bloom (1966) found that cortisone has pronounced effects on the normal development of teeth. The experimental rats received daily subcutaneous injections of 50 mg of cortisone for 8 days. Histopathologic changes were observed in pulp, predentin and dentin in these cortisone treated rats. Observed was disorganization and lowered differentiation of the odontoblasts, increased number of cells and blood vessels in the pulp, and excessive formation of a bone-like substance containing cellular and vascular inclusions in the pulp and pre-dentinal zone.

#### D. Electron Microscopy

##### 1. Developing Cells of Incisors.

The electron microscope is contributing greatly to the advancement of knowledge as indicated by the large number of investigations dealing with the fine structures of biological specimens.

Although the literature is replete with articles dealing with the ultra-structure of cells engaged in matrix formation, there are relatively few dealing with the undifferentiated cells of the developing tooth.

In a study by Pannese (1964) the epithelial origin of the enamel organ has been established by light microscopy and further analyzed by electron microscopy. The morphological characteristics of epithelial tissues are clearly demonstrable in the internal and external enamel epithelia. During the general histogenesis of the tooth, two gum-like epithelial ridges are formed from the ectodermal epithelium lining the mouth cavity. Through localized cellular proliferation in the developing jaws, these ridges grow into the underlying mesenchyme. The inner ridge or more internal ridge becomes the dental lamina and is the anlage of the ectodermal component of tooth formation. These dental lamina have areas which become thickened and these thickenings are the primordia of the enamel organs and are called enamel buds. The mesenchyme surrounding the bud shows an increased vascularity and an active cell proliferation. Densely packed mesenchymal cells appear close to the deeper surface of the enamel organ and this is the primordium of the dental papilla. The ectodermal constituent of the tooth germ will produce the enamel whereas the mesenchymal constituent gives rise to all the other tissues of the tooth.

Epithelial cells lining the concave surface of the enamel organ become

columnar and the cells of the convex surface are cuboidal. These cell layers are called the internal and external enamel epithelia. The layers are continuous at the border of the enamel organ and this area shows a high rate of cell growth. During this development, an extracellular fluid accumulates between these elements of the inner cell mass, but the loosely arranged cells remain connected by slender cytoplasmic processes. This area is now called stellate reticulum. A primitive cell contact is preserved in the thin layer between the internal enamel epithelium and the stellate reticulum proper. This layer of cells are morphologically similar to mesenchymal tissue, roundish or polyhedral in shape and is called the stratum intermedium. So in the fully differentiated enamel organ the four following constituents can therefore be distinguished: a) external enamel epithelium; b) stellate reticulum; c) stratum intermedium and d) internal enamel epithelium. Most of the internal enamel epithelium differentiates into the ameloblast layer. The mesenchymal cells of the dental papilla close to the layer of ameloblasts differentiate into odontoblasts and lay down the dentin. The remaining cells of the papilla become the pulp of the tooth.

In a study by Pannese (1964) later by Kallenbach (1966) and Sisca, Provenza and Fischlschweiger (1967) the ultrastructure of the normal cells of the enamel organ were described as follows:

The cells of the inner enamel epithelium are primarily columnar and border the basal lamina adjacent to the dental papilla. There is also a terminal pre-ameloblast which contacts and conforms to the contour of the stratum intermedium. The borders have shallow undulations but are relatively straight. The nuclei are oval in shape and appear to migrate toward the basal cell terminal



adjacent to the dental lamina. The cytoplasmic organelles include mitochondria, desmosomes, ribosomes, rough surfaced vesicles of the endoplasmic reticulum, Golgi bodies and tonofilaments. Also often seen are secretion granules and aggregates of glycogen.

The cells of the outer enamel epithelium are primarily cuboidal with numerous short processes which appear to connect and interdigitate with adjacent cells. The cytoplasmic organelles consisted of mitochondria, endoplasmic reticulum, Golgi complex, and tonofilaments.

The stellate reticulum cells varied from polygonal through stellate to spindle shape depending on the depth of the layer. Desmosomes are quite numerous in the junctioned complexes although they are not as frequently seen as in the outer enamel epithelium. The various organelles and inclusions are similar to those found in the outer enamel epithelium except there are not as many tonoelements.

The stratum intermedium consists of flattened cells which follows the contour of the basal ends of the inner enamel epithelium. Because these cells are more closely packed there are less intercellular spaces. The nuclei of the cells assume a central position and like those of the stellate reticulum occupy most of the cell body. The organelles found were the same as those in the other layers but were found in much reduced numbers.

Microvilli are a constant feature in the cell components of all strata of the tooth germ. This is confirmed by various studies; Pannese (1962); Decker (1963) and Kallenbach (1966). It has been postulated that the plicated surface membranes and their modifications, the microvilli, with the support of the appropriate organelles, participate in several extremely important cell activi-

ties. These include the cells secretory activity, the control of electrolyte concentration, cell membrane transfer by increasing surface area, and macromolecular transfer by micropinocytosis.

## 2. Differentiating Cells of Incisors.

There have been several electron microscope studies concerning the early stages of dentinogenesis and amelogenesis. These investigations have provided valuable information concerning cytodifferentiation and cytological descriptions. Jessen (1967) conducted a study on the ultrastructure of odontoblasts utilizing the technique of perfusion fixation and subsequent demineralization of adult rat incisors. He found that perfusing with gluteraldehyde improved preservation and thus he was able to observe organelles and inclusions not previously reported. His study confirmed previous observations concerning the appearance and location within cells of the classical cell organelles: nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus. The gluteraldehyde fixed tissues also revealed the presence of microtubules. Although not too much is known about their function it has been suggested that they may provide structural support to the odontoblastic process during dentinogenesis and in directing intracytoplasmic flow (Porter, et al., 1964; Behnke, 1965).

Cilia in fibroblasts from the small intestine of rats and chickens have been described by Sorokin (1962). More recently, cilia have been observed in fibroblasts in the dental pulp of the guinea pig, (Han, et al., 1965). According to Jessen (1967) the cilium also occurs in rat dental pulp and appears to be an evolutionary remnant with no mechanical or sensory function.

In the continuously growing incisor teeth of the rat, ameloblasts pass through a differentiation stage in which they are referred to as preameloblasts

before they enter the secretion stage and start to secrete enamel matrix.

Amelogenesis as described by Reith (1970) is a multiphasic process which includes several stages of cytological activity. These include the following: 1) secretory state or stage of matrix production; 2) the transitional state or stage of cell reorganization; 3) the preabsorptive stage; 4) early maturation; and 5) late maturation. His findings suggest that for the entire period of matrix formation, the enamel organ also consists of a stratum intermedium and the stellate reticulum. The stratum intermedium layer is one cell thick while the thickness of the stellate reticulum varies. The cells of the stratum intermedium and stellate reticulum have a similar ultrastructure in terms of cytoplasmic organelles. The nucleus occupies a central position within the cell. The perinuclear cytoplasm contains numerous small mitochondria, dense granules, bundles of tonofilaments, scattered profiles of granular endoplasmic reticulum, numerous free ribosomes, Golgi apparatus, microtubules and occasional smooth surfaced vesicles. While the cells of the stratum intermedium and the stellate reticulum have the same arrangement and complement of organelles, they differ primarily in that the cells of the stratum intermedium contact the basal ends of the ameloblasts and are more tightly packed than the cells of the stellate reticulum. Reith concludes that during the cell reorganization stage there is reorganization rather than a replacement of cells as indicated both by observations on the distribution of mitotic figures in enamel organ cells and by autoradiographic studies dealing with the uptake of tritiated thymidine by enamel organ cells. These studies indicate that cells needed for the production of enamel are formed before the onset of amelogenesis.

According to Garant (1972), ameloblasts are tall, highly polarized cells

joined at their proximal and distal ends by a terminal bar apparatus during their secretory state. There is also a distinct cytoplasmic extension called Tomes process which extends from the ameloblast body into the enamel matrix. The remaining portion of the enamel organ overlying the secretory ameloblasts consists of a layer of cuboidal cells called the stratum intermedium, and 3 to 5 layers of spindle to low cuboidal shaped cells that result from a compression of the stellate reticulum and outer enamel epithelium found during the earlier stages of the enamel organ. Sparsely distributed within the outer most cells of the enamel organ were mitochondria, microvilli and coated vesicles. The stratum intermedium contained a greater number of mitochondria and also contained microvilli.

In a study by Scott and Nylen (1958), a body of unknown identity was reported and described in the Golgi zone of the differentiated odontoblastic layer. They were referred to as lysosome-like bodies. In the later investigation, Jessen (1967) also reported these lysosome-like bodies and concluded they were more numerous in the Golgi zone but were also seen in other areas. He has suggested on a morphological basis that they belong to a group of lysosome-like bodies classified as storage granules.

Kallenbach (1971) described two phases of preameloblast differentiation. In the early phase the preameloblasts have many free ribosomes and microtubules. The cells elongate, Golgi vesicles move toward the predentin, and a dense material with a coarse granular texture appears between preameloblasts and within the predentin. Later in differentiation there is evidence of lysosome formation. Also preameloblast processes push through the basal lamin and invade the predentin. Odontoblastic processes then come in contact with the

apical border of preameloblasts. He also observed an electron dense material appearing in the extracellular space between preameloblasts bases and concluded it was an enamel precursor. In a later study Kallenbach (1972) has reported that there are spherical granules of an electron dense material which occur within the cisternae of the rough endoplasmic reticulum in the base of preameloblasts. It is suggested that intracisternal granules in preameloblasts and ameloblasts are caused by interference with transport of enamel proteins from rough endoplasmic reticulum to Golgi apparatus. Also in preameloblasts, these granules would play a role in a storage mechanism. Certain experimental procedures have been reported to lead to the formation of intracisternal granules. Weinstock (1970) reported that 2-3 hours after the injection of puromycin, when protein synthesis starts to recover, intracisternal granules can be seen in the rough endoplasmic reticulum of rat incisor ameloblasts. It is suggested that the effect of puromycin is due to an interruption of transport of newly synthesized protein from the rough endoplasmic reticulum to the Golgi apparatus caused either by a side effect of puromycin on energy intracellular transport or possibly by a direct effect on protein synthesis proper.

### 3. Hormonal Effects on Tissue.

Pollard (1969) investigated the cytochemical nature of ultrastructural nucleolar transformation in the vaginal epithelium of the ovariectomized mouse after local application of estradiol. Estradiol treatment induced ribonucleic acid synthesis, especially in the nucleolus but also in the nuclear chromatic region and ribosomes. These results were determined by utilizing a preferential stain for ribonucleic acids and comparing tissues from treated and untreated controls. Lias and Stumpf (1968) found RNA synthesis in the nuclei of

glandular cells in the prostate of castrated rats. This was observed throughout the nuclear chromatic region but only feebly in the nucleolar region. However, in animals injected with testosterone there was a vigorous synthesis of RNA in the nucleoli and in their vicinity in glandular cells of the prostate.

Garg, Szabo, Khandekar, and Kovacs (1971) reported ultrastructural changes caused by pregnenolone -16 $\alpha$ - carbhitrile in the liver of hypophysectomized rats. Earlier EM studies by Garg, et al. (1970) revealed that pregnenolone causes smooth surfaced endoplasmic reticulum proliferation in liver hepatocytes. In the hypophysectomized rats there was a disorganization and dilitation of the smooth surfaced endoplasmic reticulum. However, upon the treatment of these rats with pregnenolone there was proliferation and a restoration of the smooth surfaced endoplasmic reticulum in the hepatocytes of the liver in hypophysectomized rats. Stenram, et al. (1971) observed in cyclophosphamide (an alkylative agent)-treated rats, autophagic vacuoles and an apparently increased amount of lysosomes in pulp cells and odontoblasts. The Golgi system was prominent. Lamellae bodies were often seen; but also appeared in the controls. The mitochondria were sometimes slightly swollen with derangement of the cristae. The rough surfaced endoplasmic reticulum, nuclei and nucleoli showed no changes. The enamel organ showed less pronounced changes than the pulp.

## MATERIALS AND METHODS

Twelve (12) albino rats of the Sprague-Dawley strain 60 days old were utilized in this study. During the experiment, they were kept in complete darkness from 7:00 p.m. to 7:00 a.m. and in light from 7:00 a.m. to 7:00 p.m. Only the investigators entered the animal room and only at regularly scheduled visits for feeding, watering, cage cleaning and injection. Of the twelve animals, eight were adrenalectomized rats. Four of these received daily 1.0 mg injections of cortisone for a 50 day period, while the remaining four received a comparable volume of physiological saline solution. Four normal rats served as controls and they also received the daily saline injections. The rats were fed a normal pellet diet of Purina rat chow and water ad libitum. The drinking water of the adrenalectomized rats was supplemented with 1% NaCl. The animals were housed two to a cage and were further identified by picric acid marks on their fur.

Prior to the beginning of the actual investigation, a pilot project was undertaken in order to become completely familiar with the techniques involved. We also gained valuable experience in tissue sectioning and actual electron microscope use.

The techniques used in this investigation followed closely those outlined by Warshawsky and Moore (1967). This is a reproducible technique which provides sections of decalcified rat incisors for electron microscopy. Fixation is accomplished by perfusion with slightly hypertonic, neutral phosphate-buffered 2.5% glutaraldehyde for approximately 30 minutes. Throughout the rest of the

procedure isotonic neutral solutions are used. For decalcification, 4.13% disodium ethylene diamino tetra acetic acid (E.D.T.A.) for 14-21 days is employed, followed by washing in phosphate buffer for 2 days, post fixation in 1% osmium tetroxide for 4 hours and embedding in Epon. According to the authors this procedure resulted in well preserved fine structure while maintaining good tissue relationships.

In our study, few variations were employed both in the pilot project and actual investigation but were minor in nature and will be described fully as the procedure and methods are outlined. We had to sacrifice a number of test animals before acquiring good tissue fixation by perfusion. The procedure was carried out in the following manner. The animals were given ether anesthesia. The chest cavity was quickly opened and each animal was perfused first with an isotonic saline solution followed by a glutaraldehyde solution. This was done by cutting open the right atrium and simultaneously injecting by needle and syringe directly into the left ventricle. The saline was warmed to 40° C and approximately 25 ml were used over a period of about 2 minutes or until the solution from the right atrium was somewhat clear. Immediately following the saline perfusion, the glutaraldehyde solution was injected by the same procedure. It was found that perfusion with approximately 100 ml over a 20-30 minute period resulted in excellent fixation. This solution was 2.5% glutaraldehyde which was prewarmed to 40° C, slightly hypertonic and neutral phosphate buffered. A short time after beginning the glutaraldehyde perfusion, tremoring of the animal was observed and marked tissue stiffness was observed indicating good tissue fixation. The solution was carefully and slowly injected over the 20-30 minute period so that the cells and tissues would be minimally traumatized.



The head was then removed, and the skulls split midsagittally and the apical portion of the upper incisors were recovered by dissection. This tissue was kept in cold ( $4^{\circ}$  C) glutaraldehyde solution for three hours and then transferred to the decalcification solution (E.D.T.A.) previously described. This solution was also isotonic and was always kept at  $4^{\circ}$  C. The tissue was kept in this solution for a 3-1/2 week period with changes every four days.

After demineralization, the apical portion of the incisors were further trimmed using razor blades. This resulted in a piece of tissue approximately 1.5 mm square. These sections were then washed for 38 hours in a cold ( $4^{\circ}$  C) isotonic phosphate buffer with frequent changes of fluid. The washed segments were then fixed for 4 hours in 1%  $\text{OsO}_4$  at  $4^{\circ}$  C.

The sections were then dehydrated in a graded series of ethanol. The sections were in each concentration for 5 minutes, starting with a 50% solution and proceeding finally to the 100% ethanol. After dehydration, the sections were imbedded in epon and prepared for sectioning.

Thick sections ( $1\ \mu$ ) for the electron microscope were cut with diamond knives using the Reichert OmU<sub>2</sub> ultramicrotome. These sections were examined under the light microscope in order to locate and orientate the cervical loop. After finding the exact location desired, thin sections were cut and picked up on mesh copper grids, stained with uranyl acetate and lead citrate in 50% ethanol for 1/2 hour at  $60^{\circ}$  C. The grids were then washed in distilled water and examined and photographed with a Zeiss 9S electron microscope.

## RESULTS

### Observations on Control Animals

The preameloblasts or cells of the inner enamel epithelium are low columnar to cuboidal in shape. As they differentiate, they become tall columnar. In their undifferentiated state, the preameloblast layer is stratified. The preameloblasts adjacent to the stratum intermedium contact and conform to the contour of these flattened cells (Figures 3, 4 and 14).

On the opposite border, the preameloblasts are separated from the predontoblastic layer by a continuous electron-dense lamella, more properly termed the basal lamina (Figures 5, 6 and 7). There is no basal lamina present between the preameloblasts and stratum intermedium. The stratum intermedium serves as a primitive developmental cell contact between the inner dental epithelium and the stellate reticulum (Figure 8).

The preameloblasts have relatively straight, smooth borders except for occasional indentations. The nuclei of the preameloblast cells are quite large and ovoid in shape (Figure 5). Much condensed chromatin is easily seen scattered throughout the nuclear sap. The nucleolus is very prominent (Figure 9). Mitotic figures are frequently observed (Figures 8 and 10). These mitotic figures are almost always found near the stratum intermedium layer. They are very rarely seen adjacent to the basal lamina. Small desmosomes are quite often visible between adjacent cells (Figure 25). Tight junctions are also frequently seen between these preameloblasts cells (Figure 11). The cells are for the most part tightly packed, however, some extracellular spaces are seen (Figure 5).

Microvilli routinely project into these spaces from the surfaces of the pre-ameloblasts (Figure 11). Numerous mitochondria are seen, these are generally ovoid, spherical or tubular in shape and are usually rather small (Figures 7, 9 and 13). The mitochondria are made up of an outer and inner membrane. The inner membrane folds inward forming parallel folds called cristae. Free ribosomes are also liberally sprinkled throughout the cytoplasm (Figures 7 and 12). Ribosomes can be connected by a fine thread-like material called messenger RNA. These connected ribosomes become polyribosomes. Also noted are rough surfaced vesicles of the endoplasmic reticulum (Figures 7 and 15), Golgi complexes, secretion vacuoles, and several unidentified vesicles within the cytoplasm of the cells. The Golgi constituents are composed of smooth surfaced membranes, minute vesicles and vacuoles, with the membranes aligned to form parallel arrays of saccules (Figures 9 and 15).

Small homogeneous dense bodies can be seen near the basal lamina (Figure 6). These may be a secretory product; possibly enamel matrix precursor. These dense bodies closely resemble many of those found in the apical end of secretory ameloblasts (Figure 13). The basal lamina is a continuous, amorphous electron-opaque membrane. This membrane is about  $300 \text{ \AA}$  wide and thought to be composed chiefly of glycoprotein and a fibrillar material. In appearance this membrane can be straight or it can have a wavy or irregular pattern (Figure 6).

Lysosomes can be observed in the cell cytoplasm (Figures 7 and 12). These are round bodies which are membrane bound and have a very variable content. Most frequently the contents are of a heterogeneous, dense matrix. Sometimes the content consists of droplets of variable size and density. In size they are generally larger than granules and are not found as closely associated to

the basal lamina as the secretory granules.

#### Observations on Saline Treated Adrenalectomized Animals

The preameloblast cell layer of these animals was generally found to contain more extracellular space than the controls (Figure 14). This observation would seem to suggest either a reduction in cell size or an accumulation of intracellular fluid. A number of the preameloblasts of these animals displayed nuclei with several membrane bound inclusions (Figures 15, 16, 17 and 18). These structures were, many times, observed to be associated with the inner nuclear membrane (Figure 17). This suggests possible degeneration of the nuclear membrane in these adrenalectomized animals. In other instances these inclusions were found within the substance of the nucleus itself (Figures 15 and 16).

Some changes were also noted in the mitochondria. Many of the mitochondria observed were seen to be broken and distorted. This was also observed in the control tissues, however, numerically there seemed to be more in these animals than in tissues observed from the control animals. Many mitochondria showed cristae that were distorted and did not have the regular, parallel arrangement of those found in the normal mitochondria (Figures 18 and 19). In some instances the cristae also seemed to be slightly swollen (Figure 20). Frequently very elongated mitochondria were observed in comparison to any of those seen in normal samples. This conceivably can be due to the plane of section, however, while mitochondria of various lengths were seen in control animals, very few were observed of the length shown in Figure 21.

As in the control preameloblasts, granules were observed in the apical portion of the cell, however, they were not as prevalent as those noted in the

controls (Figure 22). In looking at these cells, one gets the impression that the Golgi complexes are less noticeable. This observation, however, is highly subjective and needs further study.

#### Observations on Cortisone Treated Adrenalectomized Animals

In general, the preameloblasts of these animals seem to be similar to those of the controls. Most importantly, after examining the grids of these animals, little evidence of nuclear degeneration could be found in these cells. Intracellular spaces were minimal, again suggesting a more normal cellular morphology.

The typical organelles were observed and they seemed to be equivalent to those of the control animals. However, as in the untreated adrenalectomized rats many mitochondria were observed to be quite elongated (Figure 24). Many also revealed a bizarre formation (Figure 25). However, the cristae of the mitochondria of the cortisone treated animals showed the typical parallel arrangement as noted in the controls and no evidence of swelling was observed.

The appearance and number of the granules at the area of the basal lamina seem to be equivalent to that observed in the control tissue (Figure 23).

## DISCUSSION

Our study has shown that certain ultrastructural changes take place in the preameloblast cells of the incisor in the adrenalectomized rat. Moreover, when these rats are given a replacement dosage of cortisone, these changes are not observed.

These observations can be correlated with similar studies conducted at the light microscope level. In the early work of Schour and VanDyke (1932) hypophysectomy of rats was found to cause a retardation of eruption and a cessation of amelogenesis. The administration of growth hormone greatly lessened these conditions. Goldsmith and Ross (1956) observed premature histological differentiation and eruption when cortisone was administered to postnatal rats. They also found some degeneration of the ameloblastic layer later in the treatment period.

Wellband (1961) reported a histological degeneration and atrophy of the rat enamel organ after adrenalectomy. Most affected were the stratum intermedium and stellate reticulum layers with lesser changes in the preameloblasts. However, he reported little if any histological restoration in the enamel organ when cortisone was administered at the rate of 1.5 mg daily.

Following adrenalectomy, Schour and Rogoff (1936) report degenerative changes of the enamel organ resulting in a disturbance in the calcification of the dentin and enamel of rat incisors. Another interesting correlation to our study is the one conducted by Baume et al. (1953-54). They studied the effects of thyroidectomy and hypophysectomy on the rat incisor. Following these opera-

tions they noticed reduced eruption rates, a reduction in cell differentiation, and atrophy of the preodontoblasts and inner enamel epithelium. The administration of growth hormone to hypophysectomized rats resulted in some renewal of dental tissues, but no stimulation in the eruption rate. Thyroxin accelerated the eruption rate and resulted in improved amelogenesis. Similar to our study, the administration of these hormones resulted in an improved condition of the incisor tissues and caused a maximum restoration of the enamel organ.

Although our study did not deal directly with incisor eruption, our findings would seem to support the hypothesis that cellular proliferation is the basic cause of tooth eruption; a theory put forth by Addison and Appleton (1915) Sicher (1942), Hoffman and Gillette (1964) and others.

The alterations in the preameloblasts of our adrenalectomized rats, especially in the nuclei and the mitochondria, strongly indicate that removal of this gland causes basic changes in the structure of these cells, therefore, resulting in severe interferences in the function of this renewing cell population.

Kiely and Domm (1973) has shown that adrenalectomy results in an inhibition in the number of 3H-thymidine labelled nuclei of the preameloblasts, stratum intermedium, preodontoblasts, and pulp cells in the rat incisor. Cortisone injection resulted in an increase in the uptake of the isotope to values above those of controls. Also in normal rats, Domm and Kiely (1968) have shown a greater number of colchicine arrested cells in the cervical loop of the incisor when compared to controls. These results together with those of our present study provide important information at the cellular and ultracellular level, as to the mechanism involved in the severe reduction of eruption rates

following adrenalectomy observed by a number of investigators, (Garren and Greep, 1961; Parmer et al., 1951; Domm and Marzano, 1954; Domm and Wellband, 1960). This inhibition of eruption can most likely be the result of the changes visualized in the proliferating tissues of the incisor. Likewise, the stimulation of eruption following cortisone administration can be correlated with the restoration of these tissues observed following treatment with this steroid.

Our present investigation generally agrees with the ultrastructure description of the preameloblast cells as described by Pannese (1964), Kallenbach (1966) and Sisca, Provenza and Fischlschweiger (1967). The preameloblasts are cuboidal to tall columnar and border the basal lamina adjacent to the preodontoblasts. At the other border the cells contact and conform to the stratum intermedium with no basal lamina present. The nuclei of preameloblasts are large and oval and seem to migrate away from the basal lamina. The major cytoplasmic organelles include: mitochondria, desmosomes, ribosomes, rough endoplasmic reticulum, Golgi bodies, lysosomes and granules.

Kallenbach (1971) has described an electron dense granular material within the cytoplasm of the preameloblasts adjacent to the basal lamina. He considers these dense bodies to be enamel precursor. At the moment, however, limited information is available as to the origin, final destination, composition or precise role of these granules. However, Reith (1970) also believes this granular, stippled material to be an enamel precursor secreted by the preameloblasts. If this is true, then these cells which have been considered to be a wholly progenitor population are, in fact, carrying on to a limited extent, a secretory role usually ascribed to more differentiated cells. We have noticed in light and electron microscope preparations that the greatest majority of



mitotic figures are found adjacent to the stratum intermedium. Few if any mitoses are seen near the basal lamina. This observation would seem to indicate that these more apically located cells have lost their capacity to divide and are initiating their secretory function. Kiely and Domm (1973) have also observed a greater number of 3H-thymidine labelled preameloblasts adjacent to the stratum intermedium.

Kallenbach (1971) believes a mechanism is operational whereby these granules affect the undifferentiated mesenchymal cells to become preodontoblasts. When these odontoblastic cells start secreting the dentin matrix there is an effect on the preameloblasts so that they change their polarity; i.e., the nucleus and organelles occupy specified positions in the undifferentiated cell. When this is accomplished satisfactorily, the cells then commence to secrete the enamel matrix. In his later study, Kallenbach (1972) also refers to these granules as playing a role in a storage mechanism. In our study, it is interesting to note that in the untreated adrenalectomized rats there were areas in which little of this granular material was present (Figure 22). The correlation between this observation with the other cellular effects of adrenalectomy and the eruptive process is not evident at this time, but it does seem to warrant further study and consideration.

The results of our study seem to correlate with hormonal effects on other tissues of the body. Pollard (1969) reported a restoration of ribonucleic acid synthesis after treating ovariectomized rats with estradiol. Lias and Stumpf (1968) found that castrated rats injected with testosterone showed a vigorous synthesis of RNA in the nucleoli themselves and in their vicinity in the cells of the prostate. The untreated control showed only feeble synthesis of RNA in

the nucleolar area. Similarly, Garg et al. (1970) observed the administration of pregnenolone to restore to normal the disorganized and dilated smooth surfaced endoplasmic reticulum in the hepatocytes of hypophysectomized rats.

Our study represents the first attempt to investigate the effect of adrenalectomy and cortisone treatment on the ultrastructure of rat incisor pre-ameloblasts. While our results are quite preliminary in nature, they do indicate that the integrity of these cells are seriously compromised as a result of the removal of the adrenals. Follow up studies at the electron microscope level, preferably quantitative in nature, are needed in order to firmly substantiate and hopefully add to our initial results. At the present time, however, we feel our results strongly suggest that the growing cells at the apex of the incisor are very much dependent on the secretions of the adrenals for their normal function, and that without this gland they cannot adequately provide the necessary forces needed for eruption.

## SUMMARY AND CONCLUSIONS

1. Twelve female albino rats, sixty days old, of the Sprague-Dawley strain were divided into three groups. Of the twelve animals, eight were adrenalectomized. For 50 days four of these eight rats received daily 1.0 mg injections of cortisone, while the remaining four received a comparable volume of physiological saline solution. Four normal rats served as controls and they also received the saline solution in daily injections.
2. The maxillary incisors were dissected from the animals following intra-arterial perfusion with phosphate buffered glutaraldehyde. Utilizing electron microscopy techniques, the apical portion of the incisors were decalcified, dehydrated and embedded in epon. The specimens were then sectioned using an ultramicrotome, mounted on grids, stained with uranyl acetate and lead citrate and the cervical loop area was examined with the electron microscope.
3. This study concentrated on the cells of the inner enamel epithelium, i.e., the preameloblasts. This cell layer from the control animals presented the typical normal histology as described by a number of electron microscopists. The cells are basically cuboidal to columnar in shape and present a stratified arrangement. Many of these cells contact and conform to the contour of the stratum intermedium, while others border the basal lamina adjacent to the dental papilla. The nucleus is large and oval in shape with the nucleolus usually quite prominent. In the cytoplasm, the mitochondria are round, ovoid or slightly elongated with parallel folds called cristae. Other cytoplasmic organelles and inclusions are ribosomes, rough endoplasmic reticulum, Golgi bodies, lyso-

somes, granules, vacuoles and several unidentified vesicles. Intercellular spaces are of varying size, but are minimal as adjacent membranes of cells are usually in close approximation, often resulting in junctional complexes. Numerous microvilli are seen projecting into these spaces. Small, dense granules are quite numerous adjacent to the basal lamina.

4. In comparison, the tissue of untreated adrenalectomized rats presented a number of alterations. The changes observed suggest a degenerative breakdown of certain cellular components at the electron microscope level. Noted specifically were: 1) an increase in intercellular space; 2) membrane bound nuclear inclusions sometimes associated with the inner nuclear membrane; 3) mitochondria with disarranged cristae and, in some cases, showing a possible swollen appearance, and 4) much less granular material adjacent to the basal lamina as compared to control rats.

5. The tissues examined from cortisone treated adrenalectomized animals appeared to be quite similar to the control animals. However, the mitochondria revealed some morphological changes similar to the untreated animals. Observed were mitochondria of unusual length and some with bizarre morphological configurations. The cristae, however, assumed their normal parallel appearance and no swelling seemed to be present.

6. This study correlated closely with a number of investigations that have been conducted at the light microscope level. Degeneration has been described in the developing tissues of the incisor following adrenalectomy by light microscopists. It is reasonable to conclude that these changes observed at the light microscope level, are related to the degenerative changes demonstrated in this investigation.

7. These observations lead to the conclusion that additional evidence has been presented to indicate that cell proliferation is a basic factor in controlling the eruption of the rat incisor. In addition, this study shows cortisone to play an important role in the maintenance of the developing cells of the cervical loop.

## BIBLIOGRAPHY

- Addison, W.H.F. and J.L. Appleton. 1915. The structure and growth of the incisor teeth of the albino rat. *J. Morph.*, 26:43-96.
- Anneroth, G. and G. Bloom. 1966. Structural changes in the incisors of cortisone treated rats. *J. Dent. Res.*, 45:229-235.
- Applebaum, E. and A. Seelig. 1955. Histologic changes in jaws and teeth of rats following nephritis, adrenalectomy and cortisone treatment. *Oral Surgery, Oral Medicine and Oral Pathology*, 8:881-891.
- Baume, L.J., H. Becks and H.M. Evans. 1954. Hormonal control of tooth eruption. *J. Dent. Res.*, 33:80-114.
- Behnke, O. 1965. Further studies on microtubules. *J. Ultrastruct. Res.*, 13:469.
- Bell, T. 1835. Quoted by Constant (1900).
- Berkovitz, B.K.B. 1972. The effect of demecolcine and of triethanamelamine on the unimpeded eruption rate of normal and root resected incisor teeth in rats. *Arch. Oral Biol.*, 10:937-947.
- Bryer, L.W. 1957. An experimental evaluation of the physiology of tooth eruption. *Intern Dent. J.*, 7:432-478.
- Constant, T.E. 1900. The eruption of teeth. *Third Congress Dentaire Intern.*, Paris, 2:180-194.
- Decker, J.D. 1963. A light and electron microscope study of the rat molar enamel organ. *Archs. Oral Biol.*, 8:301-310.

- Dommm, L.V. and M.L. Kiely. 1968. The effect of cortisone on mitotic activity in the rat incisor. *Proc. Soc. Exp. Biol. Med.*, 129:1912-1916.
- Dommm, L.V. and P. Leroy. 1951. The effect of daily injections of cortisone in the golden hamster. *Anat. Rec.*, 109:369.
- Dommm, L.V. and R. Marzano. 1954. Observations on the effects of certain hormones on the growth rate of the incisors of the albino rat. *Anat. Rec.*, 118:383 (Abst).
- Dommm, L.V. and W.A. Wellband. 1960. Effect of adrenalectomy and cortisone on eruption rate of incisors in young female albino rats. *Proc. Soc. Exp. Biol. Med.*, 104:582-584.
- Dustin, P., Jr. 1963. New aspects of the pharmacology of antimitotic agents. *Pharm. Rev.*, 15:449-480.
- Eccles, J.D. 1965. The effect of reducing function and stopping eruption on the periodontium of the rat. *J. Dent. Res.*, 44:860-868.
- Garant, P.R. 1962. The demonstration of complex gap junctions between the cells of the enamel organ with lanthanum nitrate. *J. Ultrastruct. Res.*, 40:333-348.
- Garg, B.D. 1971. Ultrastructural changes caused by pregnenolone-16a-carbonitrile in the liver of hypophysectomized rats. *Acta. Endocr. Suppl.*, 155:147.
- Garg, B.D., K. Kovacs and H. Seyle. 1970. Ultrastructural changes induced by pregnenolone nitrile in the rat liver. *J. Pharm. Pharmac.*, 22:872.
- Garren, L.D. and R.O. Greep. 1960. Effect of adrenal cortical hormones on eruption rate of incisor teeth in the rat. *Endocrinol.*, 66:625-628.

- Glickman, I., I.C. Stone and T.N. Chawla. 1951. The effect of cortisone acetate on the periodontal tissues of white mice. *J. Dent. Res.*, 30:461.
- Goldsmith, E.D. and L. Ross. 1956. A histochemical and histological study of the effects of cortisone on the lower incisors of fetal and postnatal rats. *Acta Endocrin.*, 22:23-41.
- Goldsmith, E.D. and S.S. Stahl. 1953. Effects of long-term cortisone treatment on the supporting dental structures of the rat. *J. Dent. Res.*, 32:699.
- Goodman, L.S. and A. Gilman. 1970. The Pharmacological Basis of Therapeutics. The Macmillan Company, New York, N.Y. pp. 1364-1369.
- Gowgiel, J.M. 1967. Observations on the phenomena of tooth eruption. *J. Dent. Res.*, 46:1325-1330.
- Han, S.S., J.K. Avery and L.E. Hale. 1965. The fine structure of differentiating fibroblasts in the incisor pulp of the guinea pig. *Anat. Rec.*, 153: 187.
- Herzberg, F. and I. Schour. 1941. Effect of the removal of pulp and Hertwigs sheath on the eruption of incisors in the albino rat. *J. Dent. Res.*, 20: 264 (Abst).
- Hoffman, R.L. and P.J. Gillette. 1964. Mitotic patterns in the developing roots of hamster molars. *Am. J. Anat.*, 114:321-340.
- Jessen, H. 1967. The ultrastructure of odontoblasts in perfusion fixed, demineralized incisors of adult rats. *Acta. Odont. Scand.*, 25:491-523.
- Kallenbach, E. 1966. Electron microscopy of the papillary layer of rat incisor enamel organ during enamel maturation. *J. Ultrastruct. Res.*, 14:518-533.
- Kallenbach, E. 1971. Electron microscopy of the differentiating rat incisor ameloblast. *J. Ultrastruct. Res.*, 35:508-531.



- Kallenbach, E. 1972. Granules in cisternae of the rough endoplasmic reticulum of preameloblasts and ameloblasts and a possible function of the rough endoplasmic reticulum in preameloblasts of rat incisor. *J. Ultrastruct. Res.*, 39:96-105.
- Kiely, M.L. 1964. The effect of cortisone on mitotic activity in the maxillary incisor of the young female rat. Unpublished Master's Thesis, The Library, Loyola University Stritch School of Medicine, Chicago, Illinois.
- Kiely, M.L. 1967. The effect of cortisone on the rate of eruption, DNA synthesis, and mitotic activity in the maxillary incisor of the female albino rat. Doctorate Thesis, The Library, Loyola University Stritch School of Medicine, Chicago, Illinois.
- Kiely, M.L. and L.V. Domm. 1973. Circadian uptake of 3H-Thymidine in the rat incisor under the influence of cortisone. *Proc. Soc. Exp. Biol. Med.*, 143: 844-849.
- Lio, S. and W.E. Stumpf. 1968. Autoradiographic evidence for the selective enhancement of nucleolar ribonucleic acid synthesis in prostatic nuclei of testosterone. *Endocrinology.*, 83:629-632.
- Main, J.H.P. 1961. Relationship between unimpeded erupted rates and blood pressure in the rat incisor. *J. Dent. Res.*, 40:1276 (Abst).
- Main, J.H.P. and D. Adams. 1966. Experiments on the rat incisor into the cellular proliferation and blood pressure theories of tooth eruption. *Arch. Oral Biol.*, 11:163-178.
- Massler, M. and I. Schour. 1941. Studies in tooth development, theories of eruption. *Amer. J. Orthodont.*, 27:552-576.

- Ness, A.R. 1964. Movement and Forces in Tooth Eruption, In: Advances in Oral Biology. ed. by: P.H. Staple, Academic Press, New York, N.Y., Vol. 1:33-76.
- Ness, A.R. and D.E. Smale. 1959. The distribution of mitosis and cells in the tissues bounded by the socket wall of the rabbit mandibular incisor. *Proc. Roy. Soc. Biol.*, 151:106-128.
- Nylen, M.U. and D.B. Scott. 1958. Electron microscopic studies of the early stages of amelogenesis. *J. Dent. Res.*, 37:22 (Abstr).
- Pannese, E. 1964. Ultrastructure of the enamel organ. *Intern. Rev. Exp. Path.*, 3:169-218.
- Parmer, L.G., F. Katonah and A.A. Angrist. 1951. Comparative effects of ACTH, cortisone, corticosterone, desoxycorticosterone, pregnenolone on growth and development of infant rats. *Proc. Soc. Exp. Biol. Med.*, 77:215-218.
- Pollard, I. 1969. Ultrastructure evidence for the stimulation of nuclear ribonuclei acid synthesis by estradiol-17b in the vaginal epithelium of the ovariectomized mouse. *J. Endocrin.*, 47:143-158.
- Porter, K.R. and L.G. Tilney. 1964. The microbutule in cell fine structure as a constant accompaniment of cytoplasmic movements. *Proc. Prague*, Vol. B: 119.
- Reith, E.J. 1970. The stages of amelogenesis as observed in molar teeth of young rats. *J. Ultrastruct. Res.*, 30:111-151.
- Schour, I. and J.M. Rogoff. 1936. Changes in the rat incisor following bilateral adrenalectomy. *Am. J. Physiol.*, 115:334-344.
- Schour, I. and H.B. VanDyke. 1932. Changes in the teeth following hypophysectomy. I. Changes in the incisor of the white rat. *Am. J. Anat.*, 50:397-

434.

- Scott, J. 1953. How teeth erupt. *Dent. Pract. Dent. Rec.*, 3:345-349.
- Shklar, G. 1963. The effect of bilateral adrenalectomy on the periodontium and alveolar bone of the albino rat. *Intern. Assoc. Dent. Res.*, 41:63 (Abstr).
- Sicher, H. 1942. Tooth eruption: Axial movement of continuously growing teeth. *J. Dent. Res.*, 21:201-210.
- Sisca, R.F., D.V. Provenza and W. Fischlschweiger. 1967. Ultrastructural characteristics of the enamel organ in the early bell stage of development. *J. Baltimore Coll. Dent. Surg.*, 22:8-27.
- Sorokin, S. 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.*, 15:363.
- Stallard, R.E. 1969. Two periodontal influences. A comparison of the effects of cortisone injections and a protein-free diet in mouse tissues. *Dent. Prog.*, 2:205-207.
- Stenram, U. and H. Nordlind. 1971. Ultrastructure observation of lysosomal activation as a possible cause of incisor malformation in cyclophosphamide treated rats. *Experimentia.*, 27:1200-1201.
- Sturman, G.D. 1957. A study of the eruption rate of the rat mandibular incisor. *Yale J. Biol. Med.*, 30:137-148.
- Taylor, A.C. and E.O. Butcher. 1951. The regulation of eruption rate in the incisor teeth of the white rat. *J. Exper. Zool.*, 117:165-188.
- Thomas, N.R. 1964. The role of collagen maturation in alveolar bone growth and tooth eruption. *J. Dent. Res.*, 43:947 (Abstr).
- Thomas, N.R. 1964. The effect of inhibition of collagen maturation on eruption in rats. *J. Dent. Res.*, 44:1159, (Abstr).

- Ulmansky, M. and S. Shapiro. 1963. Changes in rat incisors resulting from trauma on the pulp. J. Dent. Res., 42:1467-1475.
- Warshawsky, H. and G. Moore. 1967. A technique for the fixation and decalcification of rat incisors for electron microscopy. J. Histochem. Cytochem., 15:542-550.
- Weinstock, A. 1970. Cytotoxic effects of puromycin on the golgi apparatus of pancreatic acinar cells, hepatocytes and ameloblasts. J. Histochem. Cytochem., 18:875-886.
- Wellband, W.A. 1961. The effects of various endocrine disturbances on the rate of eruption and the histology of the maxillary incisor in the female albino rat. Ph.D. Dissertation, The Library, Loyola University Stritch School of Medicine, Chicago, Illinois.
- Wellband, W.A. and L.V. Domm. 1964. Effect of cortisone, amputation, and food restriction on growth of rat maxillary incisors. Proc. Soc. Exp. Biol. Med., 116:718-720.

## LEGEND

A	Ameloblast	n	Nucleolus
a	Artifact	NI	Nuclear Inclusions
BL	Basal Lamina	NM	Nuclear Membrane
CL	Cervical Loop	O	Odontoblast
D	Desmosome	P	Pulp
G	Golgi Complex	PA	Pre-ameloblast
Gr	Granules	PO	Pre-odontoblast
IS	Intercellular Space	R	Ribosome
L	Lysosome	ER	Endoplasmic Reticulum
M	Mitochondria	SI	Stratum Intermedium
MF	Mitotic Figure	SR	Stellate Reticulum
MV	Microvilli	TJ	Tight Junction
N	Nucleus	V	Vacuoles

## Plate 1

Figure 1. A low power photomicrograph of a sagittal section of a maxillary incisor showing the cervical labial loop in a young normal rat. The circumscribed area delineates the cervical loops (from Kiely, 1967). X30.

Figure 2. The cervical loop of a maxillary incisor at higher magnification. Area of labelling is approximate area where sections for electron microscopy were taken (from Kiely, 1967). X200.



Figure 1.

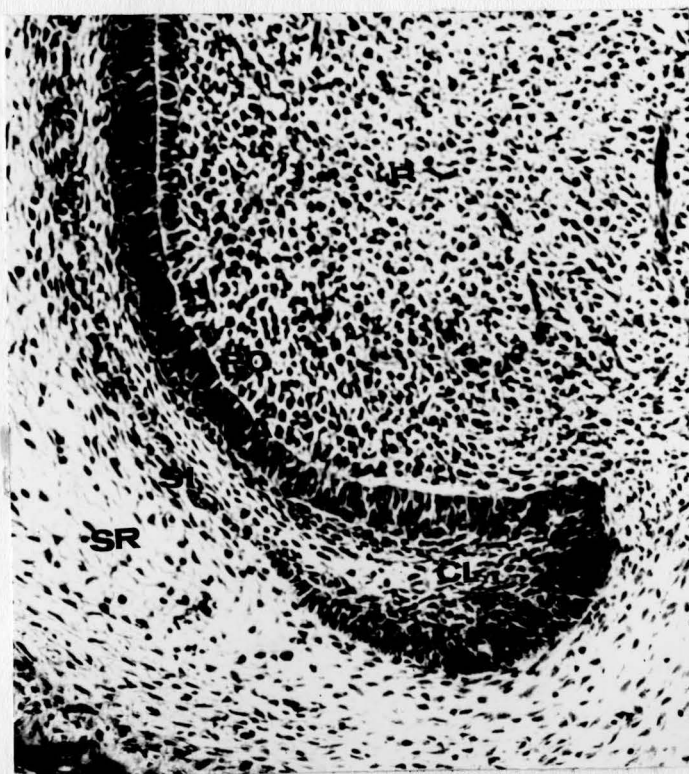


Figure 2.

## Plate 2

Figure 3. A high power photomicrograph showing the major cell layers of the cervical loop of the rat incisor. X400.

Figure 4. An oil immersion light photomicrograph showing the magnification of the cell layers shown in Figure 3. Note especially the relationship of the preameloblasts in relation to the other cell layers. Also note the mitotic figures. X1000.



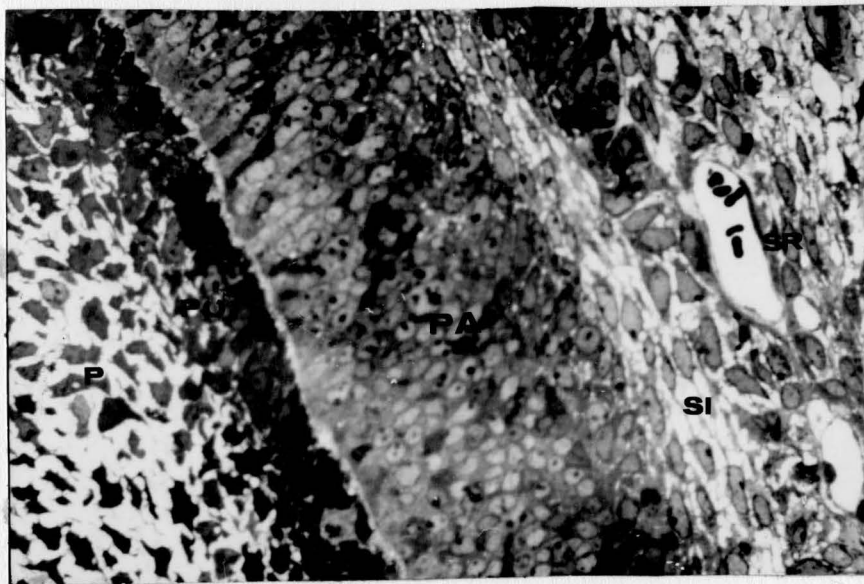


Figure 3.

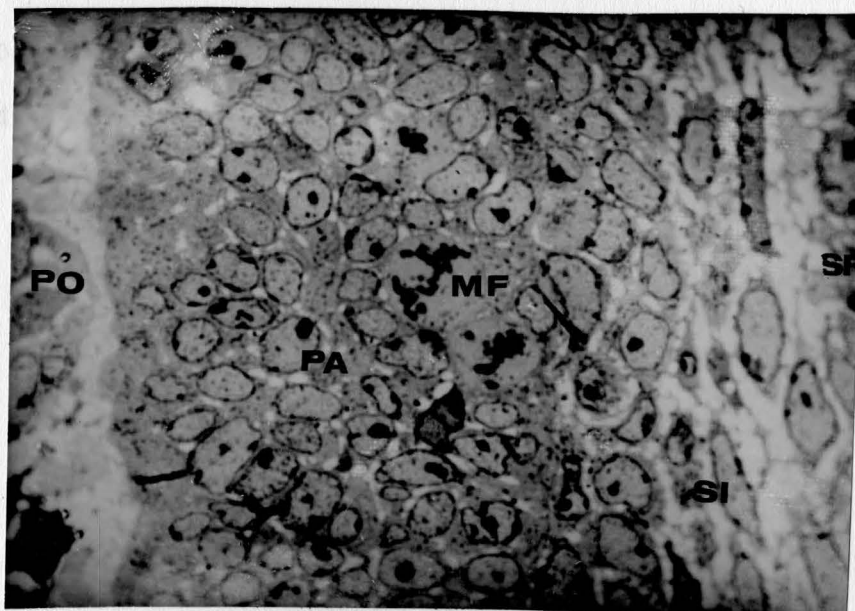


Figure 4.

## Plate 3

Figure 5. Low power electron micrograph showing the preameloblasts of a control rat. Note large ovoid nuclei and few intercellular spaces. Also note the basal lamina and the many granules adjacent to this lamina. X6650.



Figure 5.

## Plate 4

Figure 6. Electron micrograph from a control animal showing the basal lamina separating the preameloblasts and preodontoblasts. Note the many granules adjacent to the basal lamina within the cytoplasm of the preameloblasts. X6650.



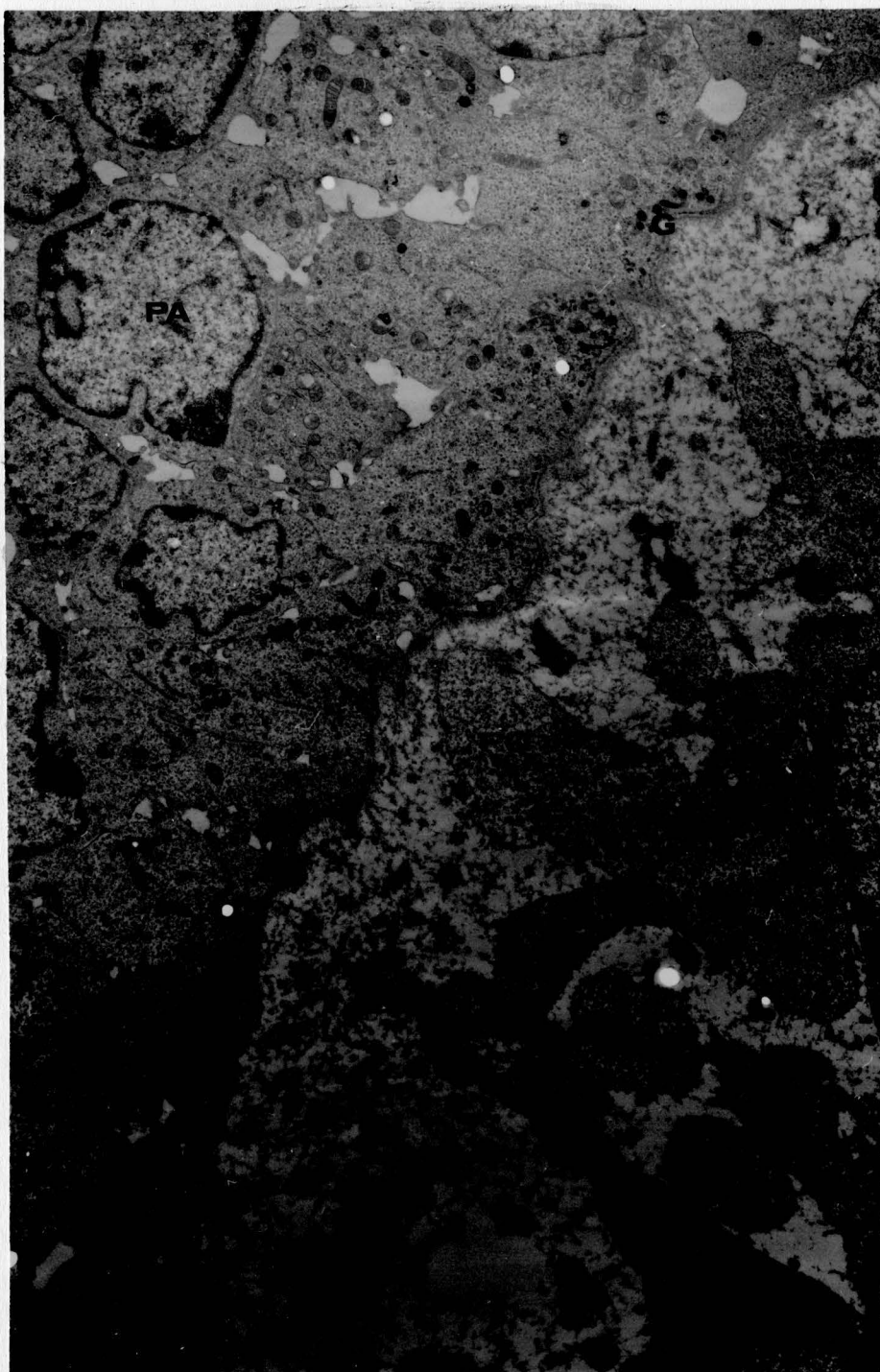


Figure 6.

## Plate 5

Figure 7. Electron micrograph from a control rat showing the width and density of the basal lamina. Note the straight, parallel cristae of the mitochondria. Also note size of granule which may be a lysosome as compared to the secretion granules. X33250.

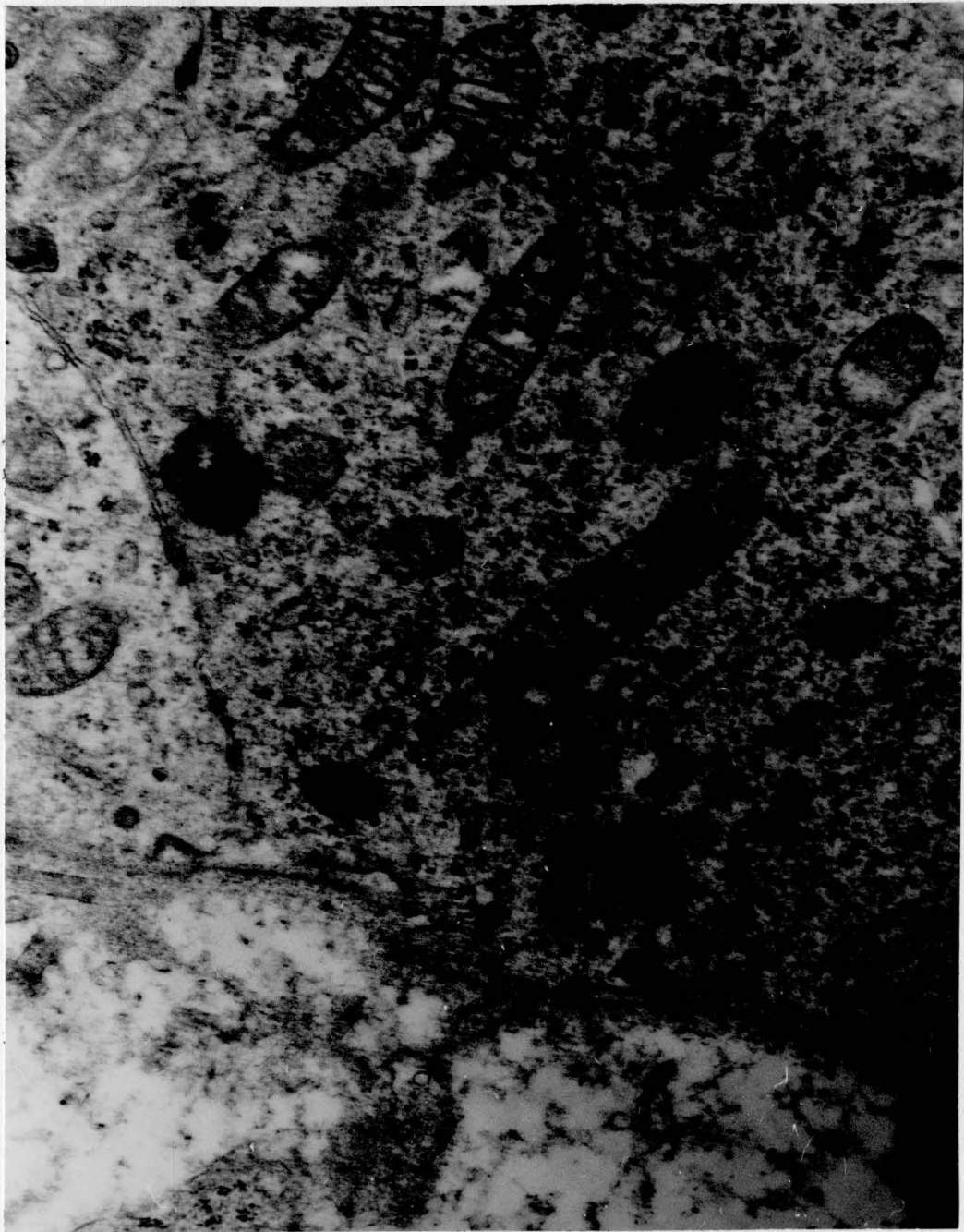


Figure 7.

## Plate 6

Figure 8. Electron micrograph of preameloblasts in a control rat.  
Note the oval shape of nucleus and presence of mitotic  
figures. X6650.





Figure 8.

## Plate 7

Figure 9. Electron micrograph of cytoplasm of normal preameloblasts with well developed Golgi complex. Note parallel membranes and vacuoles within this complex. A large nucleolus can also be seen. X33250.



Figure 9.

## Plate 8

Figure 10. Electron micrograph of control rat showing large oval nuclei and a mitotic figure. Also note basal lamina and presence of granules. X6650.



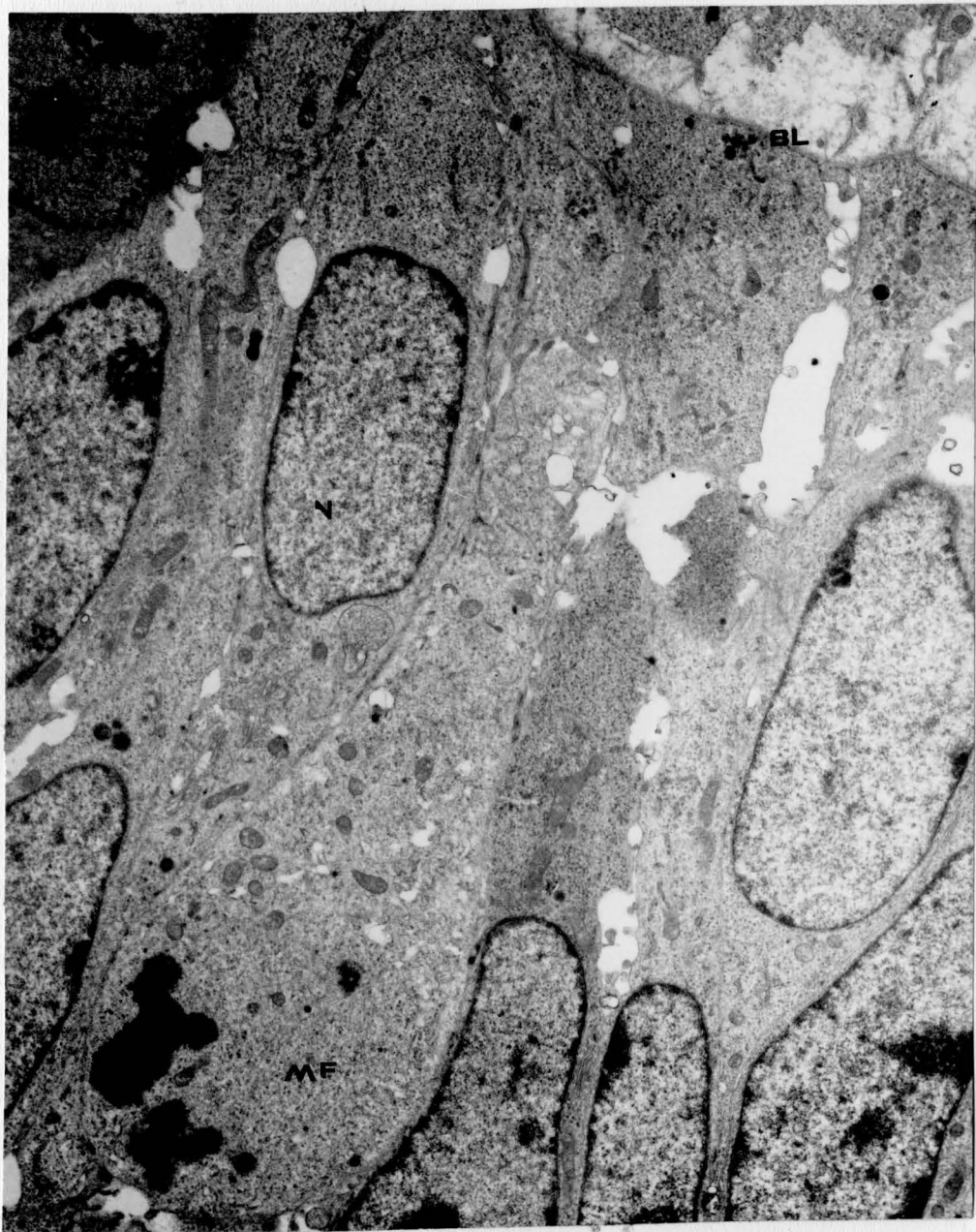


Figure 10.

## Plate 9

Figure 11. Electron micrograph of tight junction and nuclei of normal preameloblasts. Note rough endoplasmic reticulum. X33250.



Figure 11.

## Plate 10

Figure 12. Electron micrograph of several cell organelles in control animals. Note mitochondria with parallelly arranged cristae. The lysosome present shows presence of small vesicles present within it. Note the large numbers of ribosomes. X33250.





Figure 12.

## Plate 11

Figure 13. Electron micrograph of differentiated, secreting ameloblasts and odontoblasts. Note presence and size of the secretory granules as compared to those in Figure 7. X33250.

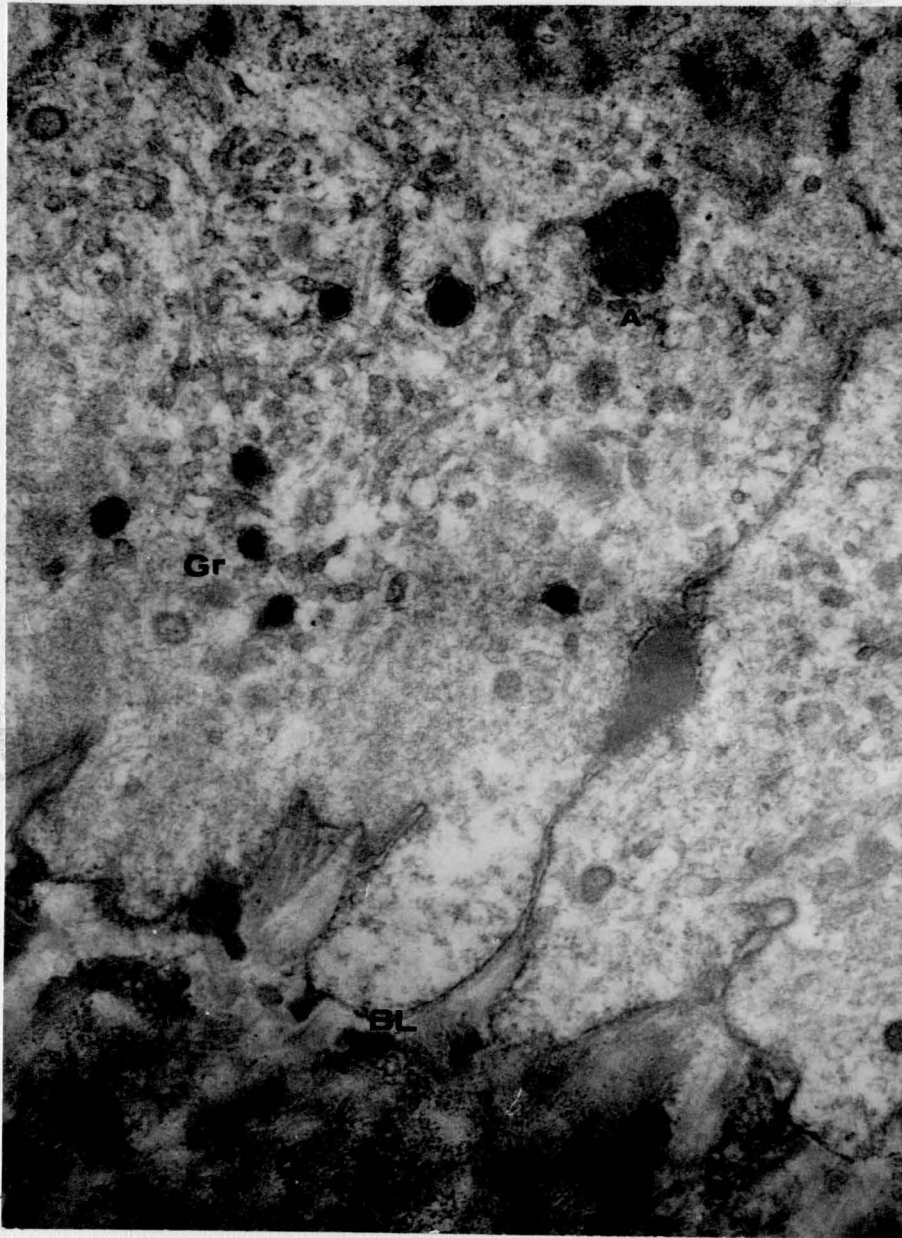


Figure 13.

## Plate 12

Figure 14. Low power electron micrograph of an untreated adrenalectomized rat showing many intercellular spaces as compared to Figure 5. Also note nuclear inclusion in small labelled nucleus. X6650.



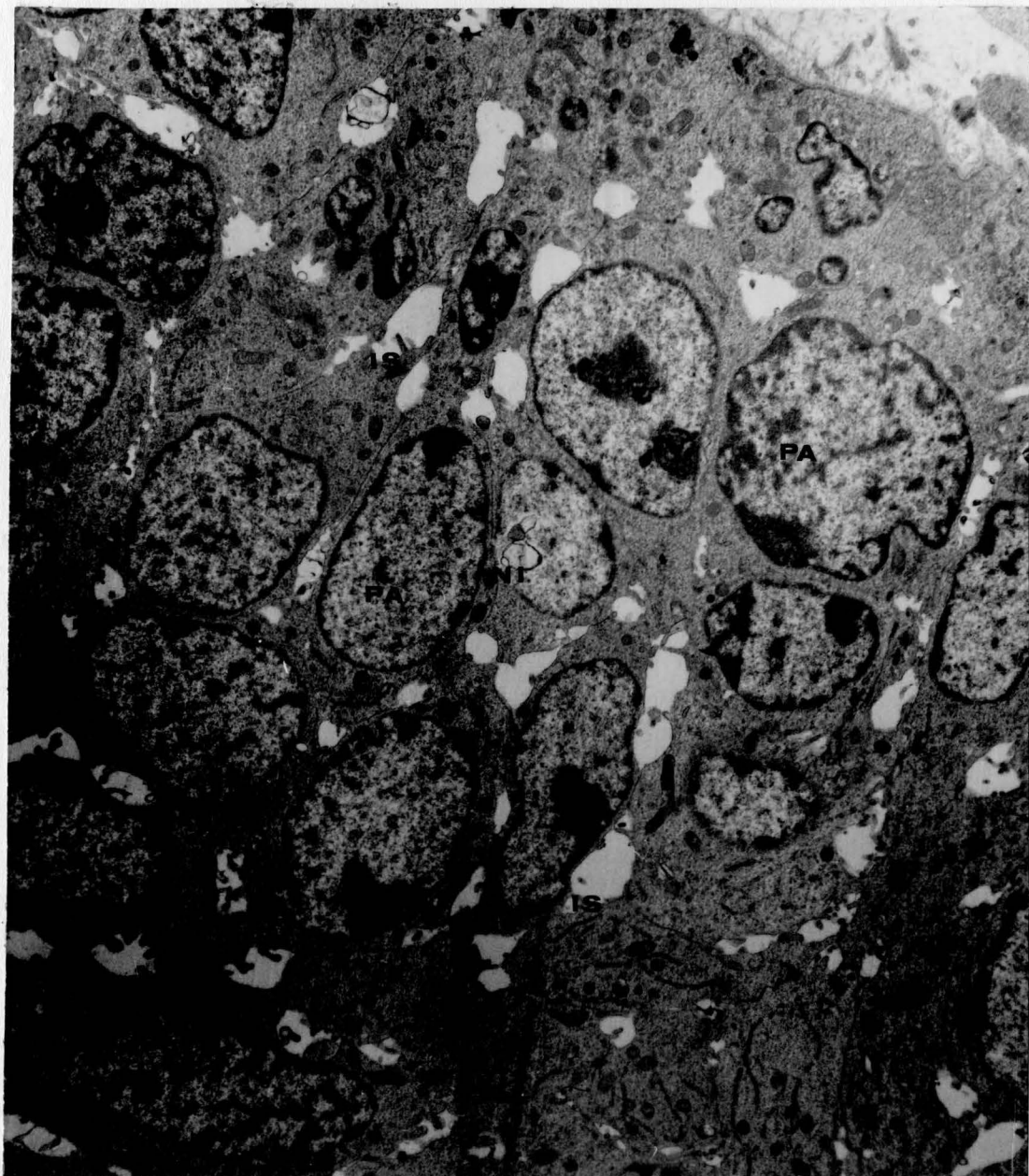


Figure 14.

## Plate 13

Figure 15. Electron micrograph of a preameloblast nucleus from an adrenalectomized rat. Note the many nuclear inclusions. A well developed Golgi complex is very prominent. X33250.



Figure 15.

## Plate 14

Figure 16. Electron micrograph of an adrenalectomized rat. Note the many nuclear inclusions. X16800.



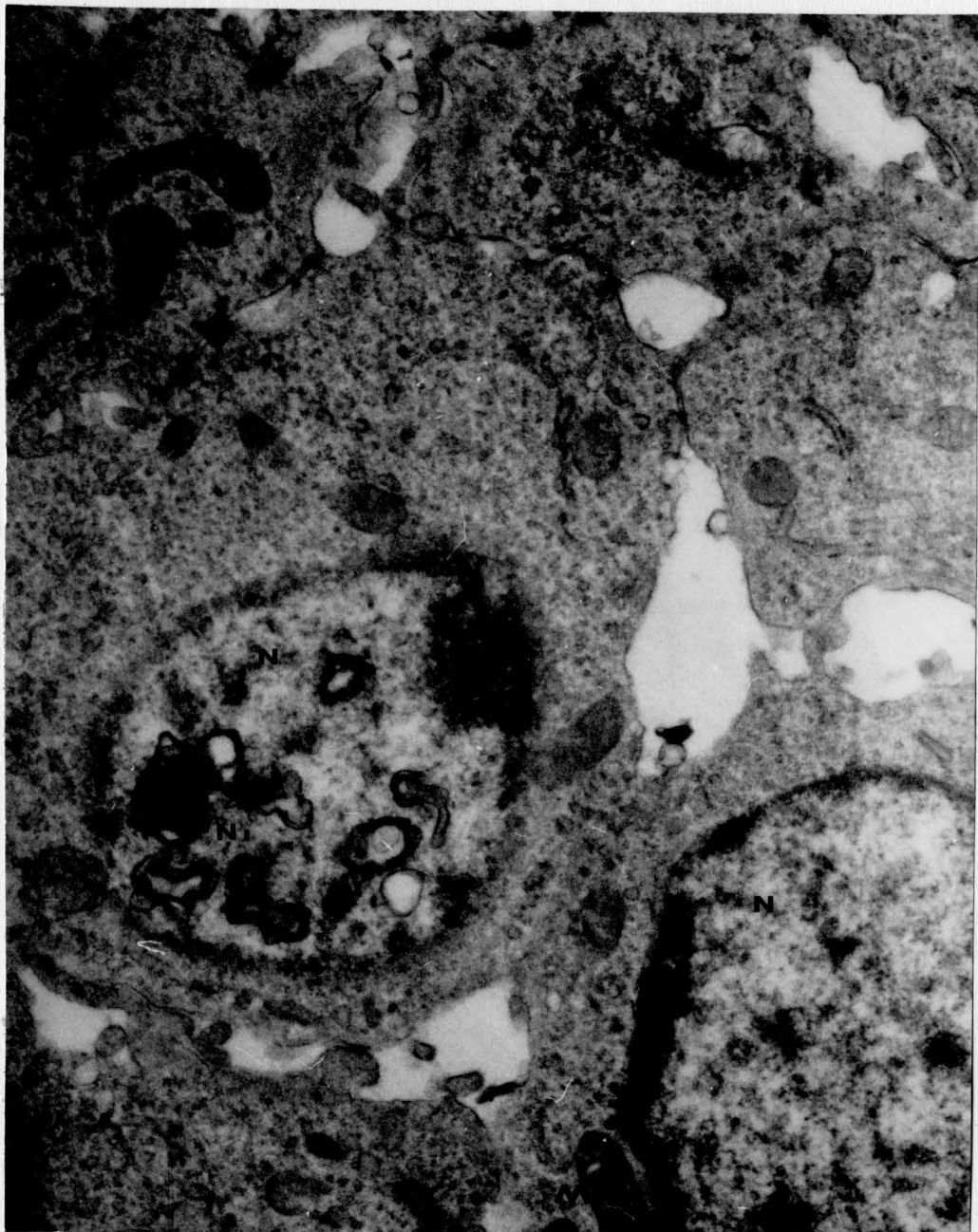


Figure 16.

## Plate 15

Figure 17. Electron micrographs of three preameloblast nuclei from an adrenalectomized animal showing nuclear inclusions.

A. X16800

B. X33250

C. X33250



Figure 17-A.

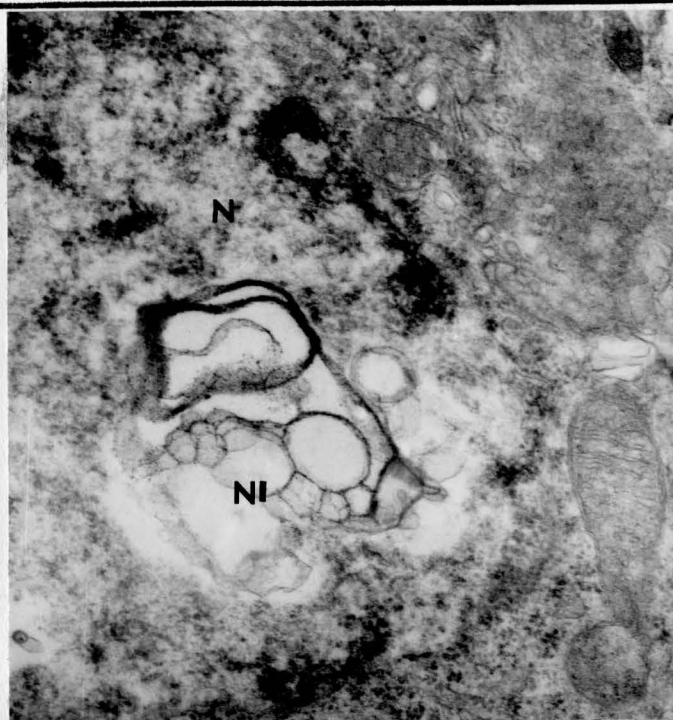


Figure 17-B.



Figure 17-C.

## Plate 16

Figure 18. Electron micrograph of a preameloblast from an adrenalectomized animal. Note the nuclear inclusions and the swollen, bizarre cristae of the mitochondria. X33250.





Figure 18.

## Plate 17

Figure 19. Electron micrograph from an adrenalectomized rat. Note the several lysosomes and vacuoles. Also note the large, bizarre mitochondria. X33250.



Figure 19.

## Plate 18

Figure 20. Electron micrograph showing some slightly swollen mitochondria cristae from an adrenalectomized rat.  
X33250.



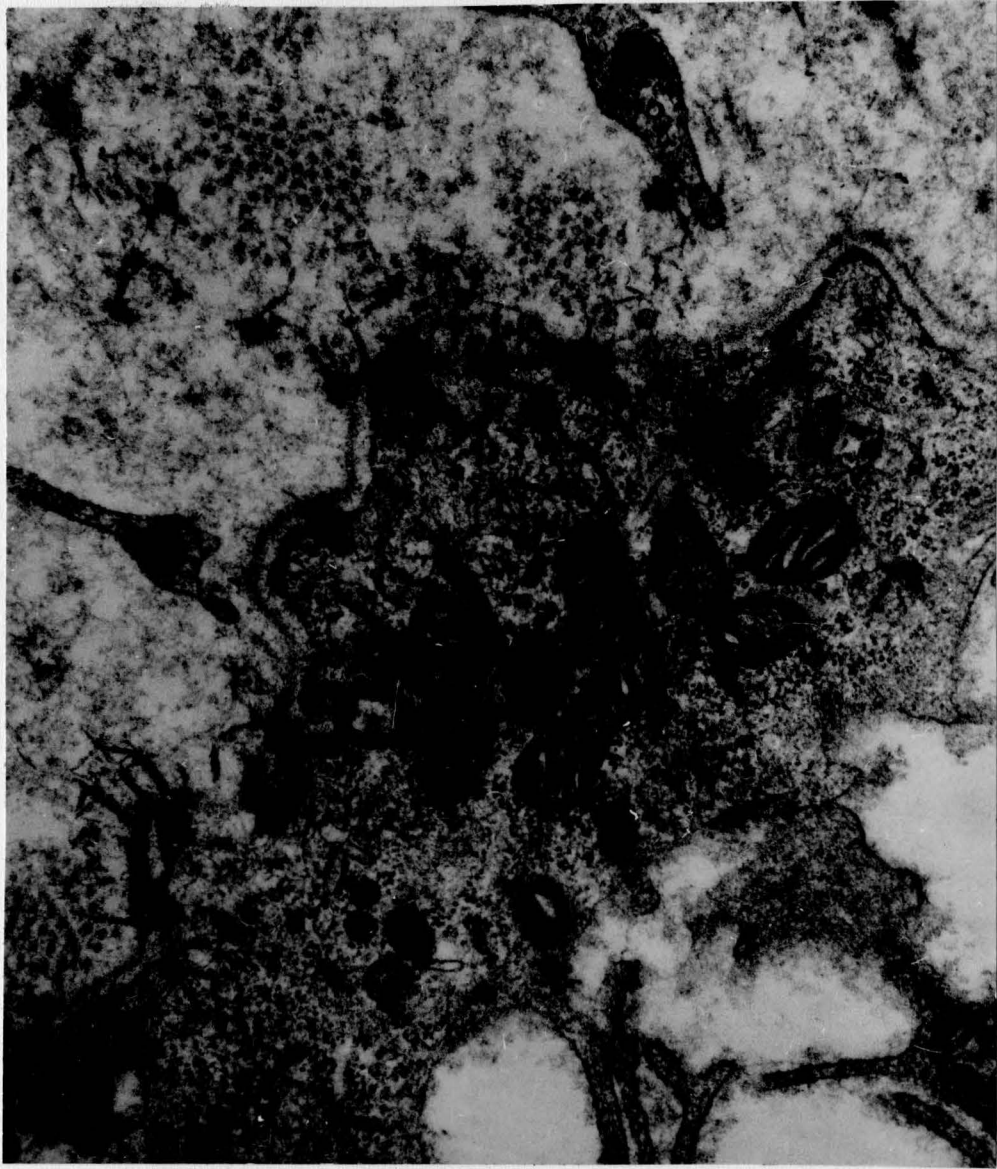


Figure 20.

## Plate 19

Figure 21. Electron micrograph of preameloblasts from adrenalectomized animal. Note the extremely elongated mitochondria. X16800.



Figure 21.

## Plate 20

Figure 22. Electron micrograph of the basal lamina from an adrenalectomized rat. Note the absence of secretion granules along the basal lamina. X16800.



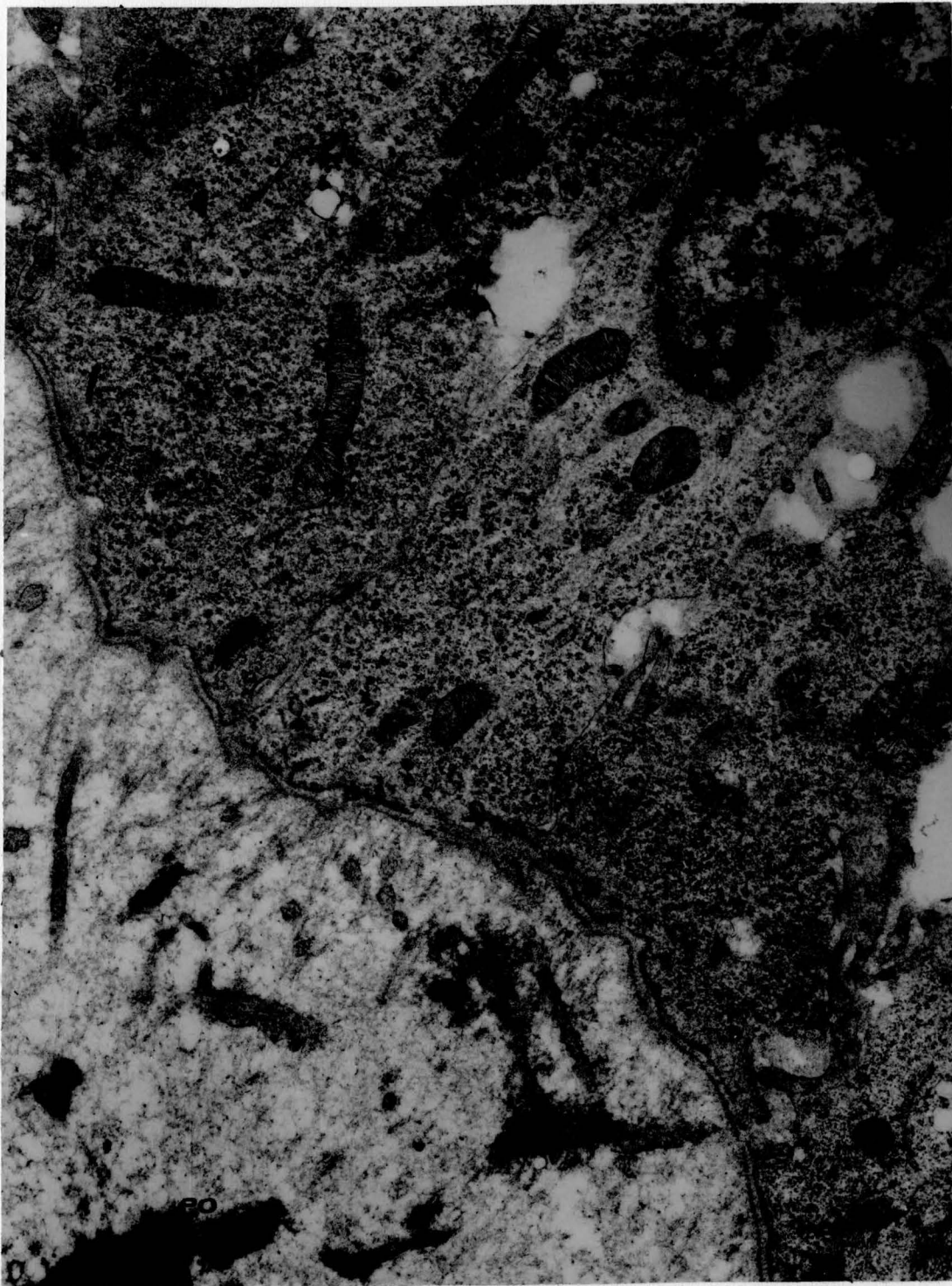


Figure 22.

## Plate 21

Figure 23. Electron micrograph of preameloblasts and preodontoblasts of a cortisone treated animal. Note the presence of many secretory granules along the basal lamina as compared to Figure 22. X6650.

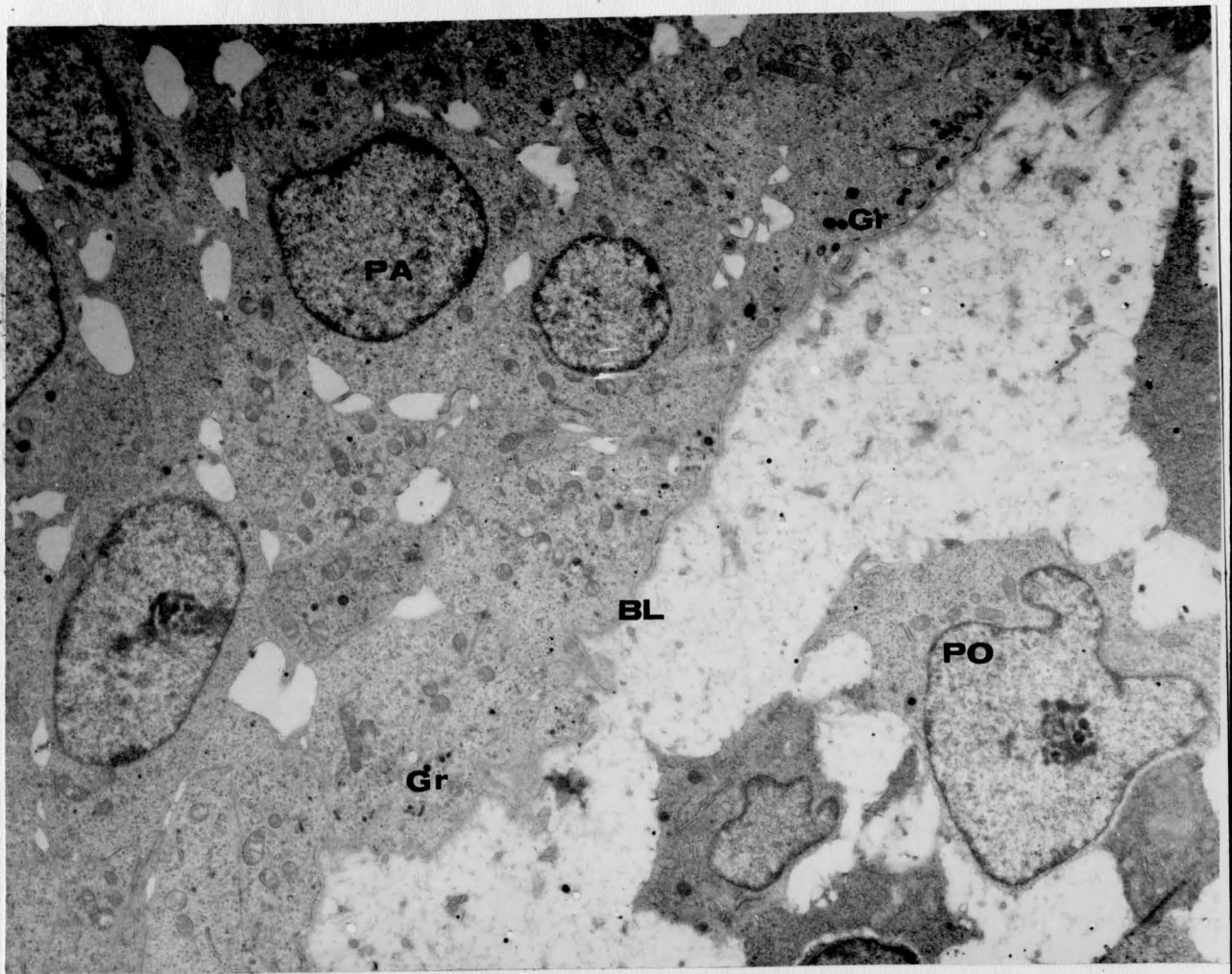


Figure 23.

## Plate 22

Figure 24. Electron micrograph of preameloblasts of a cortisone treated animal. Note the very elongated mitochondria.  
X33250.



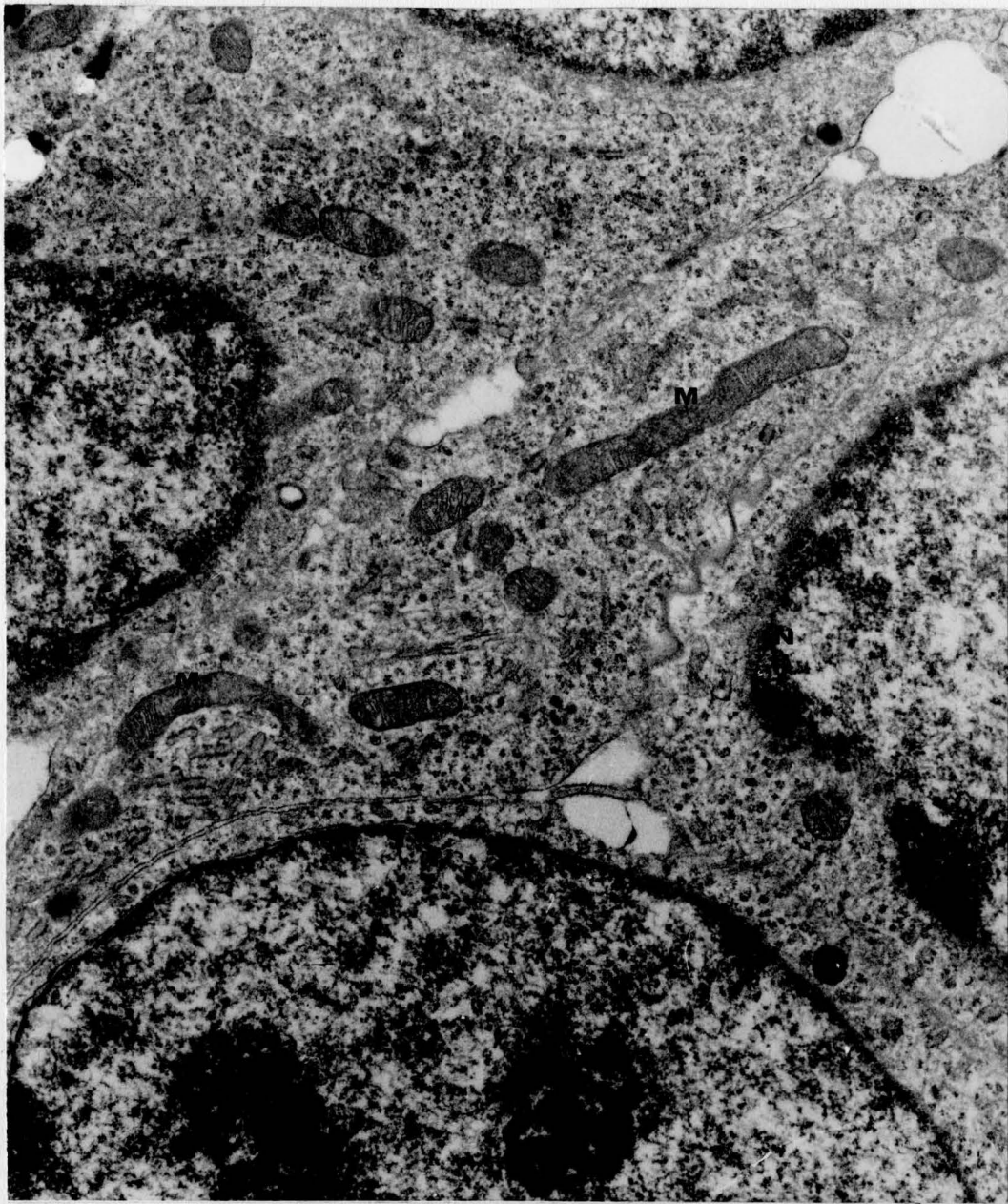


Figure 24.

## Plate 23 )

Figure 25. Electron micrograph of bizarre shaped mitochondria from cortisone treated animals. Note, however, the parallel, unswollen cristae as compared to those in Figures 18 and 20. Also note desmosome, Golgi complex and rough endoplasmic reticulum. X33250.



Figure 25.

APPROVAL SHEET

The thesis submitted by Maynard James Marshall, B.S., D.D.S. has been read and approved by the following Committee:

Dr. Michael L. Kiely  
Associate Professor, Anatomy, Loyola Dental School

Dr. Hal McReynolds  
Associate Professor, Histology, Loyola Dental School

Dr. Wayne W. Beach  
Clinical Instructor, Orthodontics, Loyola Dental School

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Date

3-19-76

Director's Signature

Michael L. Kiely Ph.D.